

In memory of my father

**CLINICAL & LABORATORY STUDIES**

**in**

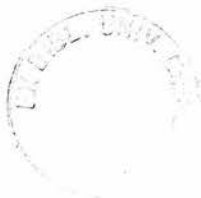
**ILEOANAL POUCHES**

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Thesis submitted to the University of Edinburgh for the degree of

**Doctor of Medicine**

**1999**





## STATEMENT

I declare that all the work contained within this thesis has been performed by myself unless otherwise stated herein. The thesis has not been submitted for any other professional qualification.

Nikolaos Evgenikos



ΟΔΗΓΟΣ ΚΑΙ ΒΟΗΘΟΣ ΜΟΙ ΓΕΝΟΙΤΟ Ο ΘΕΟΣ

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## Abbreviations

<b>5-ASA</b>	5-aminosalicylates
<b>A1AT</b>	$\alpha$ 1 Antitrypsin
<b>aIL-8</b>	anti-human IL-8 polyclonal neutralizing antibody
<b>AZA</b>	Azathioprine
<b>CD</b>	Crohn's Disease
<b>CDAI</b>	Crohn's Disease Activity Index
<b>CI</b>	Confidence Interval
<b>CLDD</b>	Centre of Liver and Digestive Disorders
<b>CRP</b>	C-Reactive Protein
<b>DMSO</b>	Dimethyl Sulphoxide
<b>EAS</b>	External Anal Sphincter
<b>ECP</b>	Eosinophil Cationic Protein
<b>EDTA</b>	Sodium Ethylene Diamine Tetracetic Acid
<b>EIM</b>	Extraintestinal Manifestations
<b>ELAM1</b>	Endothelial Leucocyte Adhesion Molecule 1
<b>ELISA</b>	Enzyme Linked Immunosorbent Assay
<b>EMG</b>	Electromyography
<b>ESR</b>	Erythrocyte Sedimentation Rate
<b>FAP</b>	Familial Adenomatous Polyposis Coli
<b>FHx</b>	Family History
<b>FMLP</b>	N-formyl-methionyl-leucyl-phenylalanine
<b>GE</b>	Neutrophil Granulocyte Elastase
<b>Hb</b>	Haemoglobin
<b>HBBS</b>	Hanks' balanced salt solution
<b>IAS</b>	Internal Anal Sphincter
<b>IBD</b>	Inflammatory Bowel Disease
<b>IC</b>	Indeterminate Colitis
<b>IgA</b>	Immunoglobulin A
<b>IgG</b>	Immunoglobulin G
<b>IgM</b>	Immunoglobulin M
<b>IHD</b>	Ischaemic Heart Disease
<b>IL-1b</b>	Interleukin -1b
<b>IL-6</b>	Interleukin-6
<b>IL-8</b>	Interleukin-8
<b>IQ</b>	Interquartile
<b>IRA</b>	Ileorectal Anastomosis
<b>LPS</b>	Lipopolysaccharide
<b>LTB4</b>	Leukotriene B4
<b>MDFC</b>	Mononuclear Derived Chemotactic Factor
<b>NBCS</b>	New Born Calf Serum

<b>NHS</b>	National Health Service
<b>NSAID</b>	Non Steroid Antiinflammatory Drug
<b>OR</b>	Odds Ratio
<b>PAF</b>	Platelet-Activating Factor
<b>pANCA</b>	Perinuclear Antineutrophil Cytoplasmic Antibodies
<b>PDAI</b>	Pouchitis Disease Activity Index
<b>PEG</b>	Polyethylene Glycol
<b>PEG+</b>	Polyethylene Glycol Electrolyte Solution with Proccessing agents
<b>PEG+/-</b>	Polyethylene Glycol Electrolyte Solution with Proccessing agents but no NBCS
<b>PLT</b>	Platelet Count
<b>PMA</b>	Phorbol Myristate
<b>PMHx</b>	Past Medical History
<b>PMSF</b>	Phenyl Methyl Sulphonyl Fluoride
<b>Pr Ptis</b>	Previous Pouchitis
<b>PTI</b>	Powell- Tuck Index
<b>QC</b>	Quality Control
<b>RIA</b>	Radioiimunoassay
<b>RIE</b>	Royal Infirmary Edinburgh
<b>rIL-8</b>	recombinant human IL-8
<b>ROS</b>	Reactive Oxygen Species
<b>RR</b>	Relative Risk
<b>SBTI</b>	Soya bean trypsin inhibitor
<b>SCFA</b>	Short Chain Fatty Acids
<b>SPECT</b>	Single Photon Emmission Computerised Tomography
<b>Tc-HMPAO</b>	Technetium-99m hexamethyl propylene amine oxime
<b>TNFa</b>	Tumour Necrosis Factor a
<b>UC</b>	Ulcerative Colitis
<b>VCAM1</b>	Vascular Cell Adhesion Molecule1
<b>WCC</b>	White Cell Count
<b>WGH</b>	Western General Hospital, Edinburgh
<b>WGLF</b>	Whole Gut Lavage Fluid

## **PRESENTATIONS & PUBLICATIONS BASED ON THIS THESIS**

### **Oral Presentations**

Whole Gut Lavage: A novel method to assess Pouchitis. Surgical Research Society. Oxford, January 1996

A study in Pouchitis. Scottish Chapter of Coloproctology of Great Britain and Ireland. Edinburgh, December 1995

Neutrophil Luminal Chemotaxis in Ileoanal Pouches. Caledonian Society of Gastroenterology. Perth, November 1996

Cytokine levels in the gut lumen in ileoanal pouches. School of Surgery Day University of Edinburgh. Edinburgh, November 1996

Luminal neutrophilia in ileoanal pouches. International FALK UK Workshop: The pelvic Ileal reservoir in Ulcerative Colitis. Oxford, April 1997

Are cytokines elevated into the gut lumen in Pouchitis? Biennial Meeting European Council of Coloproctology (ECCP). Edinburgh, June 1997

Comparison of Pouchitis Disease Activity Index (PDAI) and Moskowitz criteria for diagnosis of Pouchitis. British Society of Gastroenterology. Glasgow, March 1999



**Abstracts**

**N Evgenikos, DCC Bartolo, A Ferguson.** Whole Gut Lavage: A novel method to assess Pouchitis. Br J Surg 1996; 83(5): 693

**N Evgenikos, S Ghosh, DW Hamer-Hodges, DCC Bartolo, A Ferguson.** Whole Gut Lavage Fluid Analysis: An Objective Measure of Inflammation in Ileoanal Pouches. Immunology and Cell Biology 1997; 75 (1): A110

**S Ghosh, A Lear, N Evgenikos, A Ferguson.** Investigations of Gut Luminal Neutrophil Chemoattractants using the Polarisation Assay on Whole Gut Lavage Fluid. Immunology and Cell Biology 1997; 75 (1): A109

**N Evgenikos, DCC Bartolo, DW Hamer-Hodges, A Ferguson.** Whole Gut Lavage Inflammatory parameters in Recently Constructed Ileoanal Pouches. Gut 1997; 40(S1): A55

**N Evgenikos, DCC Bartolo, DW Hamer-Hodges, A Ferguson.** Luminal neutrophilia in ileoanal pouches. Proceed Intern FALK UK 1997; A2

**N Evgenikos, DCC Bartolo, DW Hamer-Hodges, A Ferguson.** Immunoglobulin presence in gut lumen in ileoanal pouches. Proceed Intern FALK UK 1997; A6

**N Evgenikos, DCC Bartolo, DW Hamer-Hodges, A Ferguson.** Are cytokines elevated into the gut lumen in Pouchitis? Int J Colorect Dis 1997; 12(3): 150/A133

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## Abstract

Inflammation of the ileoanal pouch, 'pouchitis', is a common complication of restorative proctocolectomy mainly observed in ulcerative colitis. Its incidence has been estimated up to 45% in some series, although there is no universal agreement between clinicians of its definition. The difficulty lies in that histopathological changes do not parallel the observed symptoms in all cases. Thus when defining pouchitis emphasis may be placed in either histology or symptoms. The aetiopathogenesis also is still largely unknown. This thesis explores the hypothesis that inflammation in ileoanal pouches has similarities to inflammatory bowel disease (IBD). Therefore methods of investigation of gut inflammation used in IBD should be readily applicable. One such method is whole gut lavage. During whole gut lavage the patient consumes a polyethylene glycol electrolyte solution until clear fluid is passed. The first clear fluid specimen passed -gut perfusate- is collected for analysis.

The pouch patients recruited in this study were first analysed clinically. Their assessment included endoscopy and pouch biopsy. The pouch patients' assessment showed an association between the inflammation of the ileoanal pouch and the extraintestinal manifestations. However, there was no such relation to backwash ileitis with ileoanal pouch inflammation. A clinical algorithm based on patients clinical features was devised to facilitate the appreciation of inflammatory events.

The whole gut lavage study performed in pouch patients showed that there is gut protein loss in ileoanal pouches similar to that in IBD. This protein loss relates to disease activity and may be used as an objective indicator of disease activity in ileoanal pouches. The protein loss is evident in the first few months of pouch function and may settle at a later stage.

The whole gut lavage method allowed measurements of haemoglobin in the lavage specimen. This whole gut lavage haemoglobin also coincides with the protein loss. The bleeding however did not relate to the protein loss in all cases.

Cytokine activity in the form of raised IL-1 $\beta$  and IL-8 in the whole gut lavage seems to accompany the gut protein loss. Luminal neutrophilia measured by whole gut lavage neutrophil granulocyte elastase relates to the IL-8 levels. This observation was initially described in whole gut lavage studies in ulcerative colitis. Luminal neutrophilia was apparent in some cases of high protein loss at a level much higher than noticed in ileostomy patients. Luminal neutrophilia again has been previously shown to be a feature of active ulcerative colitis or active Crohn's colitis but not of active small bowel Crohn's disease.

In an attempt to analyse possible chemotactic activity of the luminal contents, the method of neutrophil polarisation assay was evaluated and applied to whole gut lavage samples. The assay is based on the observation that when a culture medium is devoid of chemotactic factors, neutrophils remain spherical; with addition of chemoattractants, neutrophils acquire a polarised morphology. The use of this assay in gut fluid was the first ever attempt to use this assay in the gut. It was found that certain modifications of the lavage processing had to be made for the assay to work. It was apparent that even normal control subjects had a variable percentage of polarisation. In pouch patients the percentage of polarisation noticed was independent both from the disease activity parameters but also from the luminal or mucosal neutrophilia. This indicated that the luminal chemotaxis phenomenon as pictured by the assay was multifactorial. Chemotactic and antichemotactic factors acting together probably are responsible for this chemotactic potential.





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*‘...Λεγειν τα προγενομενα, γινωσκειν τα παρεοντα,  
προλεγειν τα εσομενα. Μελεταν ταυτα. Ασκειν  
περι τα νοσηματα, δυο, ωφελεειεν η μη βλαπτειν...’*

*Hippocratic Oration in Epidemiorum, 5th C. BC*

*‘...State the preceding, know the precepts, foresee the prospective.  
Study them all. Practice medicine, **duo, benefire, no nocere...**’*

# **CHAPTER I**

## **AIMS & APPROACH OF THESIS**

The operation of restorative proctocolectomy has been a major advance in the surgical management of patients requiring total colectomy. During the operation, a pouch of ileum is constructed and stitched to the anal muscles. Thus, a permanent spout ileostomy is avoided, and most patients with an ileoanal pouch have an acceptable degree of faecal continence.

This new surgical technique has been intensely audited, and it has become apparent that some pouch patients develop an intermittent or chronic syndrome with features of inflammation; this is generally described as pouchitis.

The current definition of pouchitis incorporates clinical, endoscopic and histological parameters. Pouchitis is mainly observed in patients who underwent colectomy for ulcerative colitis. The working hypothesis that is emerging from a number of research studies is that pouchitis represents a renaissance of inflammatory bowel disease in a susceptible individual.

Parallel to the emerging body of knowledge about pouchitis, there have been synchronous developments in the diagnosis and studies of pathogenesis of inflammatory bowel disease. In particular, gut immune events are best studied with material collected directly from the gut-biopsies and secretions- rather than by tests on blood. For example, by using fluid obtained by a new technique of whole gut irrigation, various tests of inflammation allow the investigator accurately to categorize and describe patient groups in inflammatory bowel disease.

The initial and general aim of the work I have undertaken has been to test the hypothesis of pouchitis as a form of inflammatory bowel disease, by using the whole gut lavage methodology and techniques. I have therefore studied a large series of pouch patients, firstly by documenting clinical and endoscopic features, operative, past medical, family and drug history; calculating the conventional pouch disease activity index (PDAI); subdividing the pouches into normal or inflamed groups, according to whole gut lavage fluid (WGLF) parameters; then by examining the correlation and inconsistencies within these data.

If the hypothesis that pouchitis is a form of inflammatory bowel disease is true, then the disease activity of pouchitis may be accurately assessed by gut

lavage methods in a similar fashion to what is known to happen in the inflammatory bowel diseases. Ultimately, I hoped to develop gut lavage as an objective and relatively non-invasive test for assessing pouch patients.

I then compared the results in patients with pouches and patients with ileostomies. The reservoir created in pouch patients is rapidly colonized with faecal flora, as it adapts to its new added function of a capacitance organ. My hypothesis therefore was that there will be differences in the gut lavage parameters of the two groups, because of the role of bacterial flora as triggers of gut immune reactions.

Previous investigations of gut flora in pouches have mainly been directed towards analysis of the microbial taxonomy, the corresponding metabolite changes of luminal contents and the morphology of the gut mucosa. Since this flora is not only a source of antigens, but also of peptides which are chemotactic to leucocytes, analysis of the chemotactic effect of the luminal contents would be of great interest. I therefore developed a method for measuring chemotactic activity based on neutrophil polarization. This method was used to explore chemotactic activity of gut fluids from control subjects, from patients with active and inactive ulcerative colitis, and from pouch patients with and without pouchitis.





# **CHAPTER II**

## **LITERATURE REVIEW**

**A. ILEOANAL POUCH**

**B. INFLAMMATORY BOWEL DISEASE**

## **2.A. THE ILEOANAL POUCH**

### **2.A.1. Indications-Complications-Function**

#### **2.A.1.1. Introduction**

One of the major advancements in the management of diseases requiring total colectomy has been the introduction of restorative proctocolectomy. The first series with a description of an ileoanal pouch come from Sir Alan Parks, St. Marks Hospital in London (Parks & Nicholls, 1978). Since then, few modifications of the surgical technique have appeared, with the description of the 'J' pouch by Professor Utsunomiya (Utsunomiya et al, 1980) and the 'W' pouch by Nicholls (Nicholls & Lubowski, 1987). Myers, Rothenberger and Goldberg give an analytical description of the evolution of surgical thought for anal preservation and the techniques that were used (Myers et al, 1993). The earlier development of the Kock's continent reservoir is worth mentioning. This continent ileostomy may be considered as the immediate step towards the creation of the ileoanal pouch. Many of the problems and complications of this design were common in the ileoanal pouches. This made Kock's pouches a valuable guide and a model for comparison, especially at the early days of the ileoanal pouches (Myers et al, 1993).

#### **2.A.1.2. Indications**

The indications for restorative proctocolectomy, amongst other surgical options available, are mainly Ulcerative colitis (UC) and Familial Adenomatous Polyposis (FAP) Coli (Mortensen, 1992). The Cleveland Clinic group reviewed 1005 patients who had restorative proctocolectomy over a period of 10 years. This showed that 85% of patients had a preoperative histopathologic diagnosis of UC, 8% Indeterminate Colitis (IC), 6% FAP (Fazio et al, 1995). About 1% underwent operations for other diagnoses: stage 2 (TNM classification) colon and rectal cancer, multiple cavernous haemangiomas of the colon and rectum and Hirschsprung's disease. There have been reports of use of the pouch operation

for functional bowel disorders, that is intractable slow transit constipation, when the ileorectal anastomosis was considered 'unsuccessful' (Hosie et al, 1990). However, a later report from the same group admitted poor results of this surgical treatment. Four out of eight patients in which they attempted the procedure required pouch excision. This was thought to be due to continuing feeling of incomplete evacuation and abdominal bloating. The procedure was therefore abandoned (Keighley et al, 1993).

### **2.A.1.3. Contraindications**

Old age does not seem to be a contraindication. Many operations have by now been performed on patients above the 70 years' threshold. There was no particular difference in the adverse events when compared to the younger population of pouch patients (Mortensen, 1993). Since there is a decrease of the resting and squeeze pressure with increasing age (McHugh & Diamant, 1987), special care should take place upon the selection of this group. The Edinburgh experience has been that the procedure should be contraindicated only when the preoperative sphincter function is severely compromised (Bartolo & Duthie, 1993).

Crohn's Disease (CD) is considered to be a contraindication, but recently there has been debate about the validity of this statement. Equally there is debate on the role of restorative proctocolectomy in cases of indeterminate colitis. The histological characterisation of the resected colon in the latter may be difficult. The following sections 2.A.1.5 and 2.A.1.6 discuss these controversies.

### **2.A.1.4. Complications**

#### **2.A.1.4.1. Morbidity & Mortality**

In the large reported series the complication rate of ileoanal pouch surgery has been considerable. Fazio from Cleveland Clinic reports an overall morbidity of 63% and mortality of 1% in 1005 patients, with septic complications on 7% of patients and reoperation rate of 24% (Fazio et al, 1995).

The morbidity reported by Mayo Clinic is approximately 50% (Grotz & Pemberton, 1993), while the Birmingham group reported an overall morbidity of 41% in 168 restorative proctocolectomies (Keighley et al, 1993). The early experience in St. Marks Hospital, before 1985, showed a morbidity of 45% and a mortality of 2% (Setti-Carraro et al, 1994).

It has been observed that the rate of specific complications for the procedure decreases with increase in expertise (Keighley et al, 1993). This may account to a reduction of morbidity to 30% in the most recent Mayo Clinic series (Grotz & Pemberton, 1993). Complications observed may be divided in early and late onset (described in the following sections 2.A.1.4.2 & 2.A.1.4.3).

## **2.A.1.4.2. Early Postoperative Complications**

### **2.A.1.4.2.1. Pouch Specific**

Pelvic infection, as an early complication, happened in 5% of patients with UC in the Mayo Clinic (Grotz & Pemberton, 1993). Abdominal sepsis was noticed in 6% early post operatively and this had a grave consequence as 41% of those required pouch excision eventually (Pemberton, 1993). Small bowel obstruction occurred in 13%, with 5% requiring surgical intervention (Pemberton, 1993).

Other immediate complications are pouch infarction with an expected rate of 0.1% - 3% (Fazio et al, 1995; Keighley et al, 1993), bleeding into the pouch 3%- 4% (Setti-Carraro et al, 1994; Fazio et al, 1995), and fistula formation 0.9%-1.2% (Setti-Carraro et al, 1994; Fazio et al, 1995).

### **2.A.1.4.2.2. General Complications**

General complications of chest infection, deep vein thrombosis, myocardial infarction, wound infection, urinary tract infections are encountered in almost 24% of patients (Setti-Carraro et al, 1994). Ileostomy closure has also its own morbidity; 9% of small bowel obstruction and 1% anastomotic leakage (Pemberton, 1993).

### **2.A.1.4.3. Late Postoperative Complications**

#### **2.A.1.4.3.1. Anastomotic Stricture**

A common problem post-operatively, is anastomotic stricture at the pouch anal anastomosis. The incidence of this web stenosis in most cases is higher at a later stage amounting to about 5-15% of patients (Pemberton, 1993; Fazio et al, 1995; Keighley et al, 1993). Risk factors for this complication have been singled out as the use of small diameter stapling gun, the occurrence of anastomotic disruption with resultant pelvic sepsis and the use of defunctioning ileostomy (Lewis et al, 1994).

#### **2.A.1.4.3.2 Fistula Formation**

Late complications include pouch-vagina fistula formation in 7% of female cases, as seen at a collective review of 325 women from 25 centres (Wexner et al, 1989). The overall reported incidence in the Cleveland Clinic is lower at 4%. The same authors estimate the pouch-cutaneous fistula incidence overall in 8% of the cases (Fazio et al, 1995).

#### **2.A.1.4.3.3. Intestinal Obstruction**

Intestinal obstruction continues to be a problem at the late period as well, with an estimated overall occurrence of 18%-25% (Keighley et al, 1993; Fazio et al, 1995), requiring reoperation in approximately 7%.

#### **2.A.1.4.3.4. Malignancy**

Malignancy has been observed in two cases so far (Stern et al, 1990; Puthu et al, 1992). Although the occurrence of the cancer on residual islands of rectal mucosa could not be excluded, a recent histomorphological survey found low grade dysplasia in 3 patients, who belonged to the group with permanent, near total villous atrophy, after severe pouchitis (Veress et al, 1995). One of those cases from this Swedish group had DNA aneuploidy at a biopsy from the centre of a pouch, and an adjacent biopsy showed low grade dysplasia (Löfberg et al, 1991).

#### **2.A.1.4.3.5. The Long Efferent Limb & Other Late Complications**

Late complications include intestinal malabsorption, urinary abnormalities, sexual dysfunction, superior mesenteric syndrome, adrenal insufficiency, perforation from either blunt trauma or injury to the blood supply (Santos & Thompson, 1993).

The incidence of a specific complication, the pouch obstruction due to a long efferent limb of an 'S' pouch, has almost disappeared after modification of the surgical technique and the advent of corrective procedures (Pemberton, 1993).

#### **2.A.1.5. Crohn's Disease & The Ileoanal Pouch**

Crohn's Disease can affect any part of the Gastrointestinal tract (Clark & Kumar 1994). Accordingly there is the theoretical risk that patients may develop inflammation in their pouch, if they were to have restorative proctocolectomy. Therefore there was a great reluctance over the years to offer this operation to patients with Crohn's Colitis. On certain occasions however, clinicians were faced with the problem that after the restoration with a pouch, the preoperative diagnosis of UC had to be reviewed. This was due to either the histological appearance of the colectomy specimen or to the later emergence of clinical signs reminiscent of CD.

A retrospective study of 272 patients who had restorative proctocolectomy for what thought to be UC at the time, found nine patients who could be labelled as CD. Five of them had the diagnosis made from the excised rectum on a three stage procedure. No mention was made to any features of diversion colitis in the resected specimens. The rest of the patients had their diagnosis changed because of the development of complications reminiscent of CD. Only three patients out of the nine had their pouch excised and histologically proven to have CD. The rest of the patients continued with their pouch and considerable morbidity. The authors reinforced the idea that pouch surgery has to be avoided on patients with preoperative diagnosis of CD (Deutsch et al, 1991).

The same concern was expressed in a later study, which was prospective in design with a single surgeon and pathologist participating. The differences between the complication rates of UC and CD patients did not reach statistical significance, though a 'trend' of higher percentages of pelvic sepsis, fistulas and ileoanal stenosis was observed in CD. They concluded that, when there are histological features suggestive of CD there is a higher complication rate. The medium term functional results though, may be acceptable if the pouch can be retained (Grobler et al, 1993).

The same observation was made also by the St. Marks Hospital group; the reported failure rate of 12% was due to suspected CD and perianal/pelvic sepsis (Setti-Carraro et al, 1994). Another report (Hyman et al, 1991) revealed that only one out of nine patients with preoperative diagnosis suggestive of CD had a functioning pouch amongst a group of 362 pouch procedures.

Recent reports suggest that in a selected group of Crohn's colitis, with no features of perianal or small bowel disease, restoration with a pouch may be recommended, with comparable function at five years follow up and a failure rate of 6% (Panis et al, 1996). The number of patients with CD included in the study is small (31 patients), but the follow up is reasonably long (5 years mean). The morbidity is considerable, having had 3 patients with pouch perineal fistulas and one with pouch vaginal fistula. Further prospective studies from other centres will be needed to address the same problem for an agreement to be reached.

### **2.A.1.6. Ileoanal Pouch & Indeterminate Colitis**

The controversy of whether it is advisable to perform the operation on grounds of Indeterminate Colitis (IC) still continues. Reports from Mayo Clinic (McIntyre et al, 1995) showed that the failure rate is higher in the IC group versus the UC patients (19% vs. 8%). This was in agreement with an earlier report from Vancouver, which reported a success rate of 95% for UC and 81% for IC (Atkinson et al, 1994). Another study has implicated IC with perineal complications and a greater risk of pouch loss (Koltun et al, 1991). Previously, a report from the same group in Mayo Clinic did not find any significant differences



in function, complication rate or incidence of pouchitis in these patients (Pezim et al, 1989). Admittedly though, the total number of patients' records examined in the first study was about half of those reported in the second study (514 vs. 1,232 patients). The numbers of indeterminate colitis patients were 25 vs. 71.

## **2.A.1.7. Functional Aspects Of Ileoanal Pouches**

### **2.A.1.7.1. Introduction**

The debate on surgical techniques to improve the function (i.e. storage capacity, defecation characteristics, etc.) without compromise to the 'curative' potential of the operation has been outstanding. The main areas that have been analysed were the pouch design, the type of anastomosis (hand-sewn or stapled), the height of the anastomosis, the advisability of mucosectomy, the use of medication. Several studies compared the functional outcome and the effect of the above parameters.

### **2.A.1.7.2. Frequency Of Evacuation & Continence**

The reported frequency of evacuation on the literature varies from 4-6 per day with a range of 1-20 (Fazio et al, 1995; Setti Carraro et al, 1994). Nocturnal frequency has been reported as  $1.8 \pm 1.8$  (Pena et al, 1992). In large series, seepage during the night may happen to 29% of the patients, while 10% complain of spotting during the day (Fazio et al, 1995). In the same series, 44% of patients needed to change their diet (Fazio et al, 1995). Other series have reported minor incontinence rates as high as 65% during the night and 12% during the day, requiring a night pad in 61% (Pena et al, 1992).

### **2.A.1.7.3. Physiological Assessment Of The Anal Musculature**

It has repeatedly been observed, that the resting anal pressure is decreased by over 50% post-operatively, and recovers gradually but incompletely (anal resting pressure=63 cmH<sub>2</sub>O at 18 months). Equally, the internal anal sphincter (IAS) electromyographic (EMG) activity greatly reduces post-operatively (pre-operative IAS EMG=0.51 Hz; post-operative IAS EMG=0.31 Hz at 18 months),



to recover only partly (Farouk et al, 1994). The functional length of the anal canal is significantly lower, mainly when the pouch-anal anastomosis is a low one (Deen et al, 1995). Anal endosonography reveals a reduction of the IAS thickness, with gaps and tapering (Silvis et al, 1995). Whether this is due to direct trauma, or to denervation, as some investigators have proposed, needs to be conclusively resolved. Abnormal EMG registrations of the external anal sphincter (EAS) after restorative proctocolectomy have been recorded (Emblem et al, 1989). However, the EAS voluntary contraction - 'squeeze' is well preserved and stress incontinence is an uncommon feature in these patients. It is so believed that, despite the above mentioned EMG abnormalities, no major damage is exhibited (Hultén et al, 1993).

#### **2.A.1.7.4. The Anorectal Angle**

Videoproctographic assessment of pouches has shown that the anorectal angle was not different between the various designs, at rest, during pelvic floor contraction or attempted defaecation. Its position and movement also were not different. The only parameter that influenced emptying, was the presence of an anal stricture (Kmiot et al, 1990).

#### **2.A.1.7.5. Ileoanal Pouch: Volume, Compliance & Evacuation**

Other determinants of pouch function are the volume of the pouch and the percentage of complete evacuation achieved (Nicholls, 1993; Schmidt et al, 1996; Stryker et al, 1986). The 'W' configuration (Figure 2.1) is thought to convey the higher volumes, as compared to the 'J' pouch (Figure 2.1). If the actual length of bowel is kept the same, then probably the volume achieved by the pouch depends on the availability of pelvic space (Bartolo & Duthie, 1993; Phillips, 1991). Consequently, the maximal filling pressure in one study was similar, although the 'W' pouches tolerated significantly higher filling pressures before urgency occurred (Farouk et al, 1994).

The compliance of both designs is normally comparable (Bartolo & Duthie, 1993). The evacuation of both designs is probably analogous, but worse

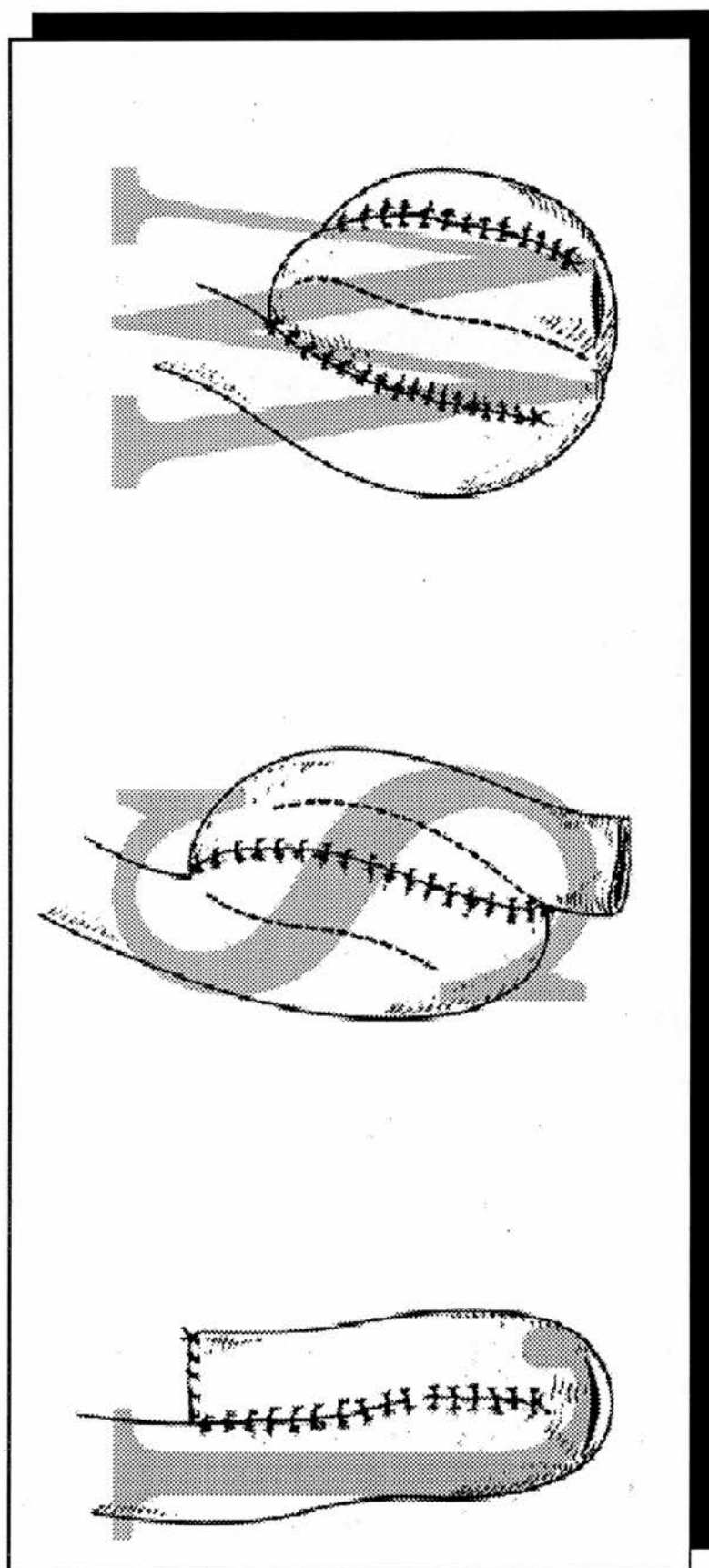


Figure 2.1 The Pouch configurations: “J”, “S” and “W”

approximately 70% vs. 90%) than in the normal rectum (Heppell et al, 1987). An interesting finding has been that, in 'J' pouches, the compliance of the pouch was higher at the lowest pressure interval (0-20 cmH<sub>2</sub>O). In pressures that are close to the threshold volume, high pressure waves are generated, which may be an important physiological parameter as compared to the normal rectum and its ability to adapt in increases of pressure (Hultén et al, 1993).

The evacuation of the 'S' pouch (Figure 2.1) is often hampered by the incidence of a long afferent limb (de Silva et al, 1991), and 40% require the use of a catheter to defaecate (Nicholls & Pezim, 1985).

#### **2.A.1.7.6. The Sensation Of The Neo-Rectum**

The sensation of neo-rectal filling and urge to defecate, as compared to the healthy rectum is probably quite different. Most patients referred to a feeling of suprapubic pressure, or borborygmi or diffuse abdominal discomfort or a feeling of deep pressure sensation in the pelvis. This measured volume of feeling was related to the functional score of the patient, but not to the sensory threshold for the urge to defaecate (Öresland et al, 1990). Others have not seen that the actual volume required for sensation in a pouch is higher than in the normal rectum (Keighley et al, 1988).

All of the above observations may indicate that storage, evacuation and sensory function of the pouch are influenced by mechanisms other than those of a normal rectum. The pouch has lost all sensory and motor connections to the pelvic pathways, with frequent, almost universal abolishment of the rectoanal inhibition reflex (Hultén et al, 1993). In spite of this the functional results in most of the patients are remarkably good.

#### **2.A.1.7.7. Poor Functional Results In Ileoanal Pouches**

Patients with poor functional outcome seem to have similar 24 hour stool output, according to one study (Groom et al, 1994). The radiological size of the pouch seemed to be equal, but the maximum tolerated volume was 290 ml, compared to 475 ml of the 'good' functional outcome group. They also seemed to have a greater number of migrating motor complexes (10 vs. 3) of the proximal

small bowel, although very few of these ever seemed to pass into the pouch (Groom et al, 1994). Other investigators have observed that patients with poor function tend to have rhythmic activity as the predominant motility pattern in their pouch, although there was considerable overlap (Levitt et al, 1992; 1994).

Incontinent patients when examined with ambulatory methodology are often noticed to have lower resting pressure in the anal canal, more prolonged anal canal relaxation, higher amplitude high-pressure waves and a non-responsive anal canal (Ferrara et al, 1994). They also seem to have rapid filling of their pouch, which in turn provokes high amplitude propulsive pressure waves (Stryker et al, 1986). The episodes of high pressure pouch waves, which often overwhelm the anal sphincter pressures, are most common during sleep (Farouk et al, 1994).

Another study using microtransducers pre- and post-operatively to record anorectal manometry, found that patients with a resting pressure of 100 cmH<sub>2</sub>O or greater than this pre-operatively, had significantly higher pressures at 12 months post-operatively. Patients with 5 or fewer bowel movements per 24 hours, had significantly higher resting anal tone. Patients who could defer defaecation for 1 hour had also higher resting anal tone (65 vs. 45 cmH<sub>2</sub>O) compared with the rest (Lindquist, 1990). Another study however, has observed that pre-operative anal manometry failed to predict clinical functional outcome in ileoanal pouches (Morgado et al, 1994). When ileostomy is constructed (before ileostomy closure), a low anal resting pressure was seen to be associated with nocturnal incontinence, whereas low pouch compliance may infer increased nocturnal frequency (Scott et al, 1989).

#### **2.A.1.7.8. Use Of Anti-Diarrhoeal Medication**

The use of Loperamide has been shown to increase resting anal pressure by approximately 20%, without affecting the squeeze pressure, the pouch volume or contractility (Hallgren et al, 1994). The anticholinergic drugs seem on the other hand to damp pouch motility, increase pouch volume and raise luminal pressures for feeling and urge (Hallgren et al, 1991). Oral Calcium supplements in one study have been shown to reduce bowel frequency in pouches compared to placebo or before treatment, while at the same time they reduced cell proliferation

(Barsoum et al, 1992). In the Minnesota study, 43% of patients required continuous anti diarrhoea medication (Pena et al, 1992). Fazio reports that 62% of his patients required the occasional or regular anti-diarrhoeal medications (Fazio et al, 1995).

#### **2.A.1.7.9. Pouch-Anal Anastomosis - Stapling & Mucosectomy**

It is believed that patients with higher pouch-anal anastomosis have better functional results, (Lewis et al, 1994) according to the Leeds group studies. However the magnitude of these differences has not been reproduced by other investigators (Hultén et al, 1993), leading to the hypothesis that the actual level of anastomosis in Leeds is even higher from what it is supposed to be, i.e. it is rather pouch-rectal (Bartolo & Duthie, 1993). This may also be the reason, why other investigators hardly ever observe any intact rectoanal inhibitory reflexes post operatively (Lindquist, 1990). If these reflexes do reappear, then the distension pressure required to elicit the response is much higher (Öresland et al, 1990).

Williams et al, have shown that stapled anastomosis are superior to the hand sewn ones, but still they also convey significant sphincter impairment (Williams et al, 1989). Seow-Choen from St. Marks Hospital on the other hand, has seen no difference in the functional outcome comparing stapled to hand sewn anastomoses with and without mucosectomy (Seow-Choen et al, 1991).

#### **2.A.1.7.10. Pouch Function & Patients' Age**

Another significant parameter of function may be related to the age of the subject. Older patients in one study had worse sphincter function compared to the younger group, but both results were within the reference range for that laboratory. The maximal tolerable pre- and post-operative pouch filling pressures, were significantly improved in both groups, indicating the advantage conferred upon the excision of a diseased non-compliant rectum (Bartolo & Duthie, 1993). This finding may explain why older patients seem to have comparable clinical functional results (Keighley et al, 1993). The Leeds group study also reflects preservation of the sensory and anal reflex function in the older age group, with

slightly inferior clinical results, which were not statistically significant (Lewis et al, 1993).

#### **2.A.1.7.11. Pouch Function In The FAP Group**

Patients who had the pouch constructed for FAP have fewer bowel movements per 24 hours and also less night time faecal spotting (Dozois et al., 1989). In this study from Mayo Clinic 97 FAP patients were compared to 758 UC patients. It is noticeable that the overall pouch specific operative complication rate (e.g. small bowel obstruction) was similar for both groups, except for pelvic sepsis, which was higher in the UC group (6% vs. 0%).

#### **2.A.1.7.12. Pouch Functional Score**

A functional score has been described incorporating most of the above clinical parameters). The score ranges from 0-15. Patients with overall good function will score 0 points, and those with poor function will score a maximum of 15 points (Table 2.1).

In this particular study (Öresland et al, 1989), parameters such as frequency of evacuation and soiling showed gradual improvement with time up to two years post-operatively, while others observed no improvement after few months (Gionchetti et al, 1995). Multivariate and step wise analyses of the above parameters have indicated that the most influential parameters are pelvic sepsis or fistula formation as well as endoanal mucosectomy (Keighley et al, 1976); other recognised parameters are maximal pouch volume and compliance (Öresland et al, 1990).

#### **2.A.1.7.13. Sexual Function & Pregnancy**

Male sexual function is generally preserved (94%), with only few cases experiencing retrograde ejaculation, decreased libido, impotence or dyspareunia (Damgaard et al 1995). In another study 8% reported improvement of their sex life, while another 8% noticed decrease of their sexual activity (Pena et al, 1992). In a review of twenty-one female patients with hystero-salpingography, 2 patients

**Table 2.1.** The functional score according to Öresland (1989). Score ranges from 0-15. Patients with overall good function will score 0 points, and those with poor function will score a maximum of 15 points.

Functional Parameters	Score 0	Score 1	Score 2
No of bowel movements daytime	≤4	5	≥6
No of bowel movements at night	0	>1/week	≥2/night
urgency *	no	yes	
evacuation difficulties**	no	yes	
soiling or seepage daytime	no	>1/week	
soiling or seepage at night	no	>1/week	
perianal soreness	no	occ.	permanent
protective pad day time	no	>1/week	
protective pad at night	no	>1/week	
dietary restriction***	no	yes	
medication ****	no	yes	
Social handicap*****	no	yes	

\* URGENCY :inability to defer evacuation >30 min

\*\* EVACUATION DIFFICULTIES :>15 min spent in toilet on any occasion during the week

\*\*\* DIETARY RESTRICTION : avoid certain items that affect pouch function

\*\*\*\* MEDICATION : continuous or occasional

\*\*\*\*\* SOCIAL HANDICAP :Not able to resume full time occupation or participate in social life



had bilateral occlusion of the fallopian tubes, 9 had unilateral, while 10 patients had tubes adhering at the bottom of the lesser pelvis (Öresland et al, 1994). In a questionnaire study from Lahey clinic with a 53% response rate, 15% reported dyspareunia, 7% faecal incontinence during intercourse, 31% menstrual problems with infertility observed in 18 patients out of 206. Fifteen patients developed pelvic cysts, 11 requiring surgery (Counihan et al, 1994). Pregnancy and childbirth were well tolerated in women with an ileoanal pouch, with a low complication rate but a higher Caesarean section rate than a historical control of women with ileostomy or Kock pouch (Juhasz et al, 1995). In the same database from the Mayo Clinic it is noticeable that from 577 women, 43 succeeded in pregnancy and delivery; 79% of the deliveries were vaginal.

#### **2.A.1.7.14. Overall Patient Satisfaction**

The post-operative patient satisfaction is high despite patients never really achieving near normal function. In the Cleveland Clinic series, 93% of patients report excellent quality of life (Fazio et al, 1995). This may be due to an improvement of the ability to work uninterrupted with a dramatic increase from 12% pre-operatively in the UC patients to 96% post-operatively (Damgaard et al, 1995). Others (Weinryb et al, 1995), compared quality of life in the same patient after ileostomy or pouch using the Psychosocial Adjustment to Illness Scale in its self report version. Restoration of bowel continuity did not yield much further improvement, as long as the initial disease was cured (Weinryb et al, 1995). In another study UC patients required at least 12 months to restore their body composition to normal (Christie & Hill, 1990).

#### **2.A.1.8. Ileoanal Pouches:**

##### **Haematological & Clinical Chemistry Aspects**

Pre-operatively patients with UC not infrequently are anaemic; this is mainly due to blood loss from the gut. Post-operatively one may expect that this problem will cease. However, in a review of 83 patients 12 months post-operatively, 4% had low values of haemoglobin compared to 22% pre-



operatively. Decreased serum iron was observed in 10% compared to 58% in the acutely ill pre-operative group. Two patients with pouch developed iron deficiency anaemia 2.5 years and 5 years later. Factors responsible may be dietary, impaired iron absorption, increased requirements or blood loss. Although the aetiology for iron deficiency is not further analysed, it does however appear that regular assessment of the full blood count in ileoanal pouch patients may be necessary (M'Koma, 1994).

In the same study, at 36 months 11% of the patients had low serum B<sub>12</sub>, while other studies have observed a variable absorption rate for B<sub>12</sub> varying from 36% to 87% (Bayat et al, 1994). The Schilling test was decreased in 36%. The main factor influencing B<sub>12</sub> absorption is the length of terminal ileum retained. The abnormal growth of the bacterial flora possibly associated with changes in the intrinsic factor, the ileal receptor for the intrinsic factor-B<sub>12</sub> complex, bacteria utilising vitamin B<sub>12</sub>, and decreased transit time due to anatomic constraints, might interfere with vitamin B<sub>12</sub> absorption (M'Koma, 1994). Folates were normal throughout the functional period.

A study on the electrolyte balance in pouches, showed that although serum electrolytes are not disturbed either in the presence of ileostomy or pouch, the ileostomy caused chronic dehydration which was compensated for by activation of the renin-aldosterone axis. The electrolyte balance becomes closer to normal after ileostomy closure following ileoanal anastomosis (Okamoto et al, 1995). However, another study found that serum chloride was lower in ileoanal pouches compared to non-operated patients or patients with ileostomy (Santavirta et al, 1991).

## **2.A.2. Pouchitis**

### **2.A.2.1. Introduction**

One of the complications that emerged after the first pouch was constructed was pouchitis. Under this term patients themselves refer to a condition that is quite similar to their previous inflammatory bowel disease, with

often bloody diarrhoea, abdominal pain, malaise and occasionally fever. Often this picture is accompanied by the re-emerge or de novo (12% in Mayo Clinic studies) appearance of extraintestinal manifestations (Mignon et al, 1995; Mortensen & Madden, 1993).

Kock first realised that the continent ileostomies are prone to inflammatory changes ("pouch-ileitis"); the same phenomenon was later discovered to hold true for ileoanal pouches (Fozard & Pemberton, 1992; McLeod et al, 1986). The first systemic approach to describe and define this phenomenon come from the St. Marks Hospital (Moskowitz et al, 1986).

### **2.A.2.2. Definition**

The definition of pouchitis went through multiple stages. Initially many clinicians, including the Mayo Clinic attendants, were inclined to diagnose pouchitis on clinical grounds only (Fozard & Pemberton, 1992). The problem of this approach is that it may not differentiate between different pouch pathologies, i.e. diarrhoea due to anastomotic stricture or long efferent limb in an 'S' pouch (Mortensen & Madden, 1993).

Subsequent work by Moskowitz, Shepherd and Nicholls standardised the syndrome as diarrhoea above six times, endoscopic findings of inflammation with a score above 4 (on a scale 0-6), and acute inflammation histopathological criteria with a score above 4 (Moskowitz et al, 1986). This triad of clinical, endoscopic and histological parameters is referred commonly as the Moskowitz criteria (Table 2.2) for diagnosis of pouchitis.

Shepherd and colleagues systematically analysed the biopsy characteristics of ileoanal pouches (Table 2.3) and categorised the acute and chronic inflammatory changes observed in ileoanal pouches (Shepherd et al, 1987). He also noticed other chronic changes in the pouch mucosa of pyloric metaplasia and changes reminiscent of mucosal prolapse. This approach however depends on good sampling, and since the endoscopic changes in pouches are often patchy, there might be a considerable sampling error (Madden et al, 1990). Furthermore, there are few severely inflamed histologically pouches, which cause minimal symptoms (Madden et al, 1990).

**Table 2.2.** The Moskowitz criteria for diagnosis of pouchitis (1986)

<b>Diarrhoea</b>	above 6 per day
<b>Endoscopic findings</b> loss of vascular pattern, granularity oedema mucosal haemorrhages contact bleeding ulceration	score one for each one present, at least four needed for diagnosis
<b>Acute histopathological criteria</b> polymorph infiltration mild moderate + crypt abscess severe + crypt abscess  Ulceration /low power field <25% ≥ 25%- ≤ 50% >50%	score 1 score 2 score 3  score 1 score 2 score 3  total <b>score≥4</b> required for diagnosis

**Table 2.3.** The Scoring system for histopathological changes in reservoir mucosa

<b>Histological feature</b>	<b>Score</b>
<b>Acute</b>	
Polymorph infiltration	
None	0
Mild + patchy infiltrate surface	1
Moderate + crypt abscess	2
Severe + crypt abscess	3
Ulceration	
None	0
Mild superficial	1
Moderate	2
Extensive	3
<b>Chronic</b>	
Chronic inflammatory cell infiltration	
None	0
Mild and patchy	1
Moderate	2
Severe	3
Villous atrophy	
None	0
Minor abnormality architecture	1
Partial	2
Subtotal	3

**Table 2. 4** The Pouchitis Disease Activity Index

Criteria	Score
<b>Clinical</b>	
Stool frequency	
Usual postoperative stool frequency	0
1-2 stools/day > postoperative usual	1
3 or more stools/day > postoperative usual	2
Rectal bleeding	
None or rare	0
Present daily	1
Faecal urgency or abdominal cramps	
None	0
Occasional	1
Usual	2
Fever (temperature >37.8°C)	
Absent	0
Present	1
<b>Endoscopic inflammation</b>	
Oedema	1
Granularity	1
Friability	1
Loss of vascular pattern	1
Mucous exudate	1
Ulceration	1
<b>Acute histologic inflammation</b>	
Polymorphonuclear leucocyte infiltration	
Mild	1
Moderate + crypt abscess	2
Severe + crypt abscess	3
Ulceration /power field	
< 25%	1
25 to 50%	2
> 50%	3

The Moskowitz criteria were also criticised by the Mayo Clinic group as being rather strict and not sensitive, and consequently a Pouchitis Disease Activity Index (PDAI) score was proposed (Sandborn et al, 1994). This index again includes clinical, endoscopic and histological parameters (Table 2.4), with a worst possible score of 18; 'pouchitis' is defined with an additive score of 7 or above. The obvious advantage of this scoring system is that it works as a continuum and not as a binary scoring system. On the other hand, its actual evaluation as a scoring system was based on comparison of data on 20 pouch patients only (Sandborn et al, 1994). No large scale prospective studies have been described upto now that used this index but one (Boerr et al, 1995). At this study 33 patients with ileoanal pouch were characterised clinically, endoscopically, and histologically, with subsequent calculation of the PDAI. All these parameters were individually correlated to the faecal  $\alpha_1$ -antitrypsin. It was evident from the figures that the PDAI provided neater correlation. In another study, faecal  $\alpha_1$ -antitrypsin clearance, as a marker of protein loss, was abnormal in few pouch patients (Fiorentini et al, 1987); one may therefore hypothesise that the PDAI may be describing the same phenomenon as gut protein loss in the pouch.

As the experience grew, it became apparent that other patients may suffer only one or two self limiting episodes of diarrhoea and ill health, but upto 17% of patients may get a continuous state of ill health with waxing and waning episodes of disease (Fozard & Pemberton, 1992).

### **2.A.2.2. Pouchitis In FAP**

The main parameter that influences the appearance of the syndrome of pouchitis is the original diagnosis for which the colectomy was performed (Fozard & Pemberton, 1992). Up to now very few cases of pouchitis have been described globally in the FAP group of patients. In his 9 year review of results on 168 restorative proctocolectomies, Keighley described 3 out of 26 FAP pouch patients who were diagnosed clinically and endoscopically to have pouchitis. All had single episodes of pouchitis (Keighley et al, 1993).

One of these patients was fully investigated with histological confirmation of the diagnosis. He had an abnormal indium-labelled granulocyte scan and

increased radioactivity in a 4 day faecal collection. Following metronidazole treatment for a month both clinical, endoscopic, histological and radionuclear parameters returned to normal (Kmiot et al, 1990).

Fazio from the Cleveland Clinic group observed pouchitis in 5 out of 62 FAP patients over a period of 10 years. No information though is given as to how the diagnosis was made and whether these patients had single or multiple episodes of pouchitis (Fazio et al, 1995).

Pouchitis was described in 6% of 63 FAP patients over a period of 40 months follow up, data obtained from the Mayo Clinic Database. The diagnosis was based only on clinical symptoms and response to antibiotic treatment, since the follow up was done over the telephone (Lohmuller et al, 1990).

### **2.A.2.3. Pouchitis In UC: Incidence & Prevalence**

Pouchitis is relatively common in UC, in contrast to familial adenomatous polyposis coli, accounting for upto 30% of the patients in one report (Fozard & Pemberton, 1992). This incidence rises with time after pouch creation. The actual figures of prevalence and incidence of pouchitis differ between centres, according to the criteria used for definition, the vigor of the follow up and also the length of follow up (Sandborn, 1994).

Recent reports from the Mayo Clinic suggest a cumulative risk of developing pouchitis in the 1st, 5th and 10th year as 15%, 36% and 46% respectively (Sandborn, 1994). A review of results on 1005 patients by the Cleveland clinic group suggested this to be 1% as an early complication, and 22.5% as a late complication (Fazio et al, 1995). The Birmingham group mentions an incidence of 18% (Keighley et al, 1993), while the St. Marks Hospital group refers to 45% had at least one episode of pouchitis, out of 66 patients reviewed post-operatively (Setti-Carraro et al, 1994).

#### **2.A.2.4. Risk Factors In Pouchitis**

Extraintestinal manifestations, pre- or post-operatively, seem to be more prominent amongst the patients who develop pouchitis (Lohmuller et al, 1990). Patients reported pyoderma gangrenosum, erythema nodosum, polyarthritits, ankylosing spondylitis, sacroileitis, uveitis, episcleritis. Patients with sclerosing cholangitis are at an even higher risk according to a later report from the same Mayo Clinic group (Penna et al, 1996). The figure given for pouchitis in sclerosing cholangitis is 63% as compared to an overall 32% incidence during a 12 year retrospective study of 1097 patients.

The presence of perinuclear antineutrophil cytoplasmic antibodies (pANCAs), identify the group of pouchitis patients who seem to have continuous symptoms (Sandborn et al, 1995). An earlier study from Birmingham, showed that pANCAs persist in the sera for two years after restorative proctocolectomy. From the 39 pouch patients who were recruited, five had pANCA in their serum and all five were patients with previous episodes of pouchitis (Patel et al, 1994).

A recent report from Oxford has revealed that smokers have fewer episodes of pouchitis, a finding in common with UC (Merrett et al, 1996). There was no association of smoking with other adverse outcomes of pouch surgery.

The presence of backwash ileitis in the resection specimen was examined in association with pouchitis (Gustavsson et al, 1987). In a retrospective study, it was revealed that pouchitis developed only in two of 15 patients with backwash ileitis and in 18 of 116 patients without ileitis. In total 20 patients had pouchitis for the two year study period. An association of pouchitis with the extent of the disease has been described; pouchitis being more common in pancolitis. However, no referral to different treatment patterns has been made (Mikkola et al, 1995); furthermore, this observation has not been confirmed by other studies (Samarasekera et al, 1995).

Repeated efforts in an attempt to correlate pouchitis with a missed diagnosis of CD have not been proved fruitful (Subramani et al, 1993). There has been a report that relates pouchitis to the pouch design, pouchitis being more common in 'J' pouches (Hewett et al, 1995).



## **2.A.2.5. Aetiology Of Pouchitis**

### **2.A.2.5.1. Introduction & Histological Appraisal**

The aetiology of the syndrome is unknown. It has been observed that a number of pouch patients manifest some inflammatory changes at endoscopy, at least in some parts of their pouches. Biopsies from pouches from different areas may vary, but invariably in 87% of the patients some elements of chronic inflammation are present, while acute inflammation may appear in 30% (Moskowitz et al, 1986). Biopsies from the posterior aspects of the pouch tend to show more inflammatory changes. This may be the result of a more prolonged contact of faeces with the bowel mucosa posteriorly (Shepherd et al, 1993).

Apel et al (1994), in a prospective study, looked at the early morphological changes of the histological picture in pouches from their creation onwards. They reported that soon after creation, pouches are infiltrated by neutrophils and eosinophils, with a mild degree of villous atrophy and Paneth's cell hyperplasia. These features remain fairly stable after six months, except for a greater degree of mononuclear infiltration, a progressive increase in the degree of eosinophilic inflammation and a new higher level of crypt epithelial kinetics. The partial transition to colonic mucin phenotype remains also stable.

The chronic inflammatory infiltrate that is observed in most of the pouches consists of lymphocytes, plasma cells, eosinophils and histiocytes (Meuwissen et al, 1989). Moskowitz has observed that the histological grading of acute inflammation is related to the sigmoidoscopic features of inflammation as well as to the frequency of defaecation; the histological inflammation could not be correlated either to the pouch design or to the percentage of evacuation (Moskowitz et al, 1986).

### **2.A.2.5.2. Partial Colonic Metaplasia**

The above described elements of chronic inflammation are commonly accompanied by varying degree of villous atrophy, at times giving a picture of partial colonic metaplasia (Mortensen & Madden, 1993).

The composition of mucus may also change in pouches, in some, attaining more colonic type sulphomucin (Corfield et al, 1992).

However, other investigators showed that the ileal glycoprotein synthesis, assessed by a glucosamine synthetase assay, does not differ in pouches, inflamed or not (Winslet et al, 1993). On the other hand, changes in goblet and columnar cells have been observed in pouches with acquirement of some colonic characteristics (Campbell et al, 1994).

In some pouches the changes of chronic inflammation attain great severity; this is known as the 'group C' (Setti Carraro and Nicholls, 1994), who invariably get chronic pouchitis. A similar study in Sweden, has shown that patients with chronic pouchitis have subtotal or total villous atrophy and some of them also display low grade dysplasia (Veress et al, 1995). Subsequently, advice for surveillance of this particular group has been issued (Shepherd, 1995).

### **2.A.2.5.3. Bacterial Ecology In Ileoanal Pouches & Pouchitis**

Over the years, there have been considerable efforts to associate pouchitis with some bacterial species resulting in an intense debate. Nicholls originally described that pouches with histological inflammation had higher aerobic counts (Nicholls et al, 1981). This was confirmed later at another study (Ruseler-van Emden et al, 1994), which showed a decreased ratio of anaerobes to aerobes, less *bifidobacteria* and anaerobic *lactobacilli* and more *Clostridium perfringens* in pouchitis. Others have seen no differences in faecal bacterial counts between pouchitis or non-pouchitis patients (Kmiot et al, 1993).

Ileoanal pouches were compared with ileostomies; pouches seem to have a greater ratio of anaerobes with more *bacteroides* and *bifidobacteria* (Nasmyth et al, 1989; Luukkonen et al, 1988) or of facultative aerobes (McLeod et al, 1994). *Bacteroides* were also implicated with the advent of villous atrophy (Nasmyth et al, 1989). One study reported that in pouchitis there was no increase in carriage of adhesive *Escherichia coli*, which is in disagreement with observations in UC (Lobo et al, 1993).

No one particular micro-organism has been implicated in the pathogenesis of pouchitis. Routine faecal cultures in clinical practice do not yield any

recognised intestinal pathogen (Nasmyth & Williams, 1993). In spite of that, metronidazole, an antianaerobic antibiotic, seems to control pouchitis, at least during an acute attack. However, the use of metronidazole in chronic unremitting pouchitis does not seem to influence the histological or the endoscopic appearance of the pouch, only partly reducing the diarrhoea (Madden et al, 1994). Metronidazole has been reported to have an effect on cell mediated immunity (Mortensen & Madden, 1993).

Onderdonk and McLeod in an electron microscopy study have demonstrated that 47% of pouch mucosa biopsies have intramural bacteria, mainly facultative aerobes, as compared to 14% in ileostomies (McLeod et al, 1994). Intramural bacteria increase, though not significantly, in pouchitis.

If a particular micro-organism cannot be held responsible for pouchitis, then there may be two possibilities. One hypothesis is that in pouchitis, the disturbed bacterial ecological system produces a bacterial metabolite that causes direct mucosal injury; or alternatively a bacterial antigen comes in close contact with the mucosa and triggers inflammation.

#### **2.A.2.5.4. Pouchitis & The Short Chain Fatty Acids Theory**

Short Chain Fatty Acids (SCFA), a product of anaerobic bacterial fermentation of dietary fiber, are thought to be trophic for the colonic mucosa, and probably for the ileal mucosa as well (Mortensen & Madden, 1993).

It is also known that SCFA stimulate terminal ileal motility, when introduced into the lumen. In pouches, however, there seemed to be an inverse relation of the total SCFA concentration per day and the stools per day (Ambroze et al, 1993). This may relate to the current finding of lower SCFA concentration in pouchitis (Sagar et al, 1995; Clausen et al, 1992). Sandborn from Mayo Clinic has reported such differences only in a subgroup of sclerosing cholangitis patients with pouchitis (Sandborn et al, 1995; Sandborn et al, 1993).

The SCFA level and composition may be influenced not only by the bacterial taxonomy (Macfarlane et al, 1992), but also by the availability of fermentable saccharides (Clausen et al, 1992) and by the pH of the environment (Ruseler-van-Emden et al, 1994). It has been shown that pouch effluent is

more alkaline in pouchitis; in alkaline conditions more mucin can be degraded, with a possible adverse effect on pouch mucosa integrity ((Ruseler-van-Emdben et al, 1994). Furthermore, the percentage of pouch evacuation may be important in determining the levels of SCFA (Nasmyth et al, 1989; Sagar et al, 1992).

SCFA have been disappointing in controlling inflammation, reducing only cell proliferation in UC pouches when utilised as a therapeutic manipulation for treatment of pouchitis (Tonelli et al, 1995; de Silva et al, 1989); in another study SCFA were only partly successful (Cutler, 1992).

#### **2.A.2.5.5. The Bile Acid Theory**

The situation with bile acid absorption is equally obscure. Hill and Owen have observed lower levels of conjugated bile acids in pouchitis (Shepherd et al, 1989), but their method has been criticised for not standardising the diet or the period post creation of pouch. Others have observed no such differences (Sandborn et al, 1995). Nasmyth showed that the bacterial metabolism of primary conjugated bile acids was higher in pouches than in ileostomy (Nasmyth et al, 1989). Salemans showed that the reabsorption of conjugated bile acids was impaired in pouches (Salemans et al, 1993). In spite of these differences, studies have concluded that the construction of a pouch facilitates restoration of a near normal bile acid profile (Kelly et al, 1993). Distinct patients, however, have impaired bile acid absorption and may be at risk of increased bile acid loss (Hosie et al, 1993).

Utilisation of jejunum as neorectum in dogs after total colectomy has shown that if the terminal ileum is spared from contact with faecal fluids, the bile acid absorption is totally normal (Juhasz et al, 1995).

#### **2.A.2.5.6. The Ischaemic Theory Of Pouchitis**

Another theory to elucidate pouchitis has been based on the observation of reduced blood flow in pouchitis, measured by laser Doppler flowmetry (Hosie et al, 1989). However, the histological findings of pouchitis do not resemble ischaemia, furthermore overt ischaemia of pouches presents itself early and in a dramatic fashion (Mortensen & Madden, 1993).

### **2.A.2.5.7. Pouchitis As A Form Of Inflammatory Bowel Disease - The Immunological Theory**

Pouchitis may be considered as reappearance of inflammatory bowel disease (IBD) (Banerjee, 1994). This hypothesis has been based on the many common elements in the natural history, pathology, response to treatment and evidence from the complications (Banerjee, 1994)

Studies of mucosal biopsies in pouchitis have shown increased levels of cytokines IL-1 $\beta$ , IL-6, IL-8 with undetectable TNF $\alpha$  in one study (Gionchetti et al, 1994), and increased TNF $\alpha$  in another (Patel et al, 1995). There were no differences in the cytokine gene polymorphism examined in patients with pouchitis (Roussomoustakaki et al, 1995). Similar findings of raised levels of IL-1 $\beta$ , IL-6, IL-8 in IBD were demonstrated in colonic biopsies of involved and non-involved IBD mucosa (Nassif et al, 1996).

Leukotriene LTB $_4$  is increased in pouchitis along with prostaglandin PGE $_2$  (Gertner et al, 1994). Platelet Activator Factor (PAF) - acether and its metabolite lyso-PAF were also increased in stools from pouchitis patients, when compared with normal pouches (Chaussade et al, 1990). The role of PAF and the eicosanoids is well described in IBD, with phospholipase-A $_2$  having a central pivotal role (Nassif et al, 1996).

The RFD9 $^{+}$  macrophages (epithelioid and tingible body macrophages), that are found in active IBD and not in infective colitis, are commonly seen in pouchitis (de Silva et al, 1991; Gionchetti et al, 1992).

The intra-epithelial lymphocyte count is paradoxically low, similar to the one in colon and it does not increase even in pouchitis (de Silva et al, 1991). The CD4:CD8 ratio of T cells is not significantly different in pouchitis compared with normal pouches (de Silva et al, 1991).

The mucosal permeability in pouches is increased, a finding described in IBD (Koltun et al, 1993). The lympho-plasmacellular infiltrate in pouchitis is characterised by an abundance of IgA and IgG bearing cells, with few IgM cells, while IgE could not be detected (Meuwissen et al, 1989).

#### **2.A.2.5.8. The Axonal Necrosis Theory**

Electron microscopy studies showed that a fair number of pouchitis biopsies appear to have axonal necrosis, in a similar way as in CD; replicate biopsies taken are also positive for bacteria, mainly facultative aerobic and intramural. At the same study an association of axonal necrosis and mast cell and eosinophil secretion was noted. All these findings are irrespective of the original diagnosis of UC or FAP (Dvorak et al, 1993).

#### **2.A.2.6. The “Objective” Assessment Of Pouchitis**

Two methods have been described for objective assessment of pouchitis: the measurement of faecal a-1-antitrypsin (Boerr et al, 1995) and the <sup>111</sup>In-labeled autologous granulocyte scan with faecal collections for four days (Kmiot et al, 1993).

The authors for the a-1-antitrypsin test reported sensitivity and specificity of 80% and 97% respectively. However, the PDAI was used for comparison, which is yet to be formally evaluated. On the other hand, this study clearly demonstrated that gut protein loss, seen in IBD, is also observed in pouchitis (Boerr et al, 1995).

The diagnosis of pouchitis in the <sup>111</sup>In-labeled autologous granulocyte scan and faecal collection study is obscure. Although clinical, histological and endoscopic parameters were studied, it seemed that the diagnosis of pouchitis was based solely on endoscopy. In spite of this weakness the authors calculated a 70% sensitivity and 100% specificity for the four hours scan. The four days faecal collection had an increased sensitivity of 90% and a specificity of 88%. An important observation may be made from this study: neutrophilia in pouchitis is concentrated in the mucosa as in IBD. Neutrophils do migrate into the gut lumen at a later stage and present themselves in the faecal collections (Kmiot et al, 1993).



### **2.A.2.7. Treatment Of Pouchitis**

The treatment of pouchitis has explored the utilisation of antibiotics. Apart from metronidazole, antibiotics such as Ciprofloxacin, Augmentin, Vibramycin have been used (Mortensen & Madden, 1993). Some patients however may require long term treatment so there is always the fear of the resultant drug toxicity. Metronidazole has been successfully utilised in a study as a local irrigation with very low blood levels (Nygaard et al, 1994). It has been suggested that patients, who do not respond to metronidazole, belong to the IC group and not to UC group (Rauhet al, 1991).

Systemic or local steroids have been used as well as 5-aminosalicylates (5-ASA) derivatives (Mortensen & Madden, 1993). Budesonide suppositories have been effective in spite of relapses when discontinued (Belluzzi et al, 1992).

Butyrate suppositories have been tried with moderate success, while glutamine substitution was more promising (Cutler, 1992). The theory behind these approaches was that both SCFAs and glutamine are considered to be trophic for the pouch mucosa. Other investigators found no improvement with SCFA enemas (Tonelli et al, 1995).

Ten out of twelve patients with pouchitis in one study did improve with the use of Bismuth carbomer enemas (Gionchetti et al, 1995).

Cyclosporin enema in one patient with unremitting pouchitis improved the clinical and endoscopic parameters of the disease (Winter et al, 1993).

Allopurinol, a xanthine oxidase competitive inhibitor that curtails free radical production, has been used successfully in a study to terminate episode of acute pouchitis, or prevent pouchitis from recurring in 50% of the cases (Levin et al, 1992).

A trial of octreotide in two patients with unremitting diarrhoea (but not pouchitis), did not show any improvement (Sagar et al, 1994).

### **2.A.2.8. The Prognosis Of Pouchitis**

The prognosis of pouchitis depends on the pattern of presentation. 39% of all pouchitis patients present with self limited disease that confers no problems as it responds promptly to antibiotics (Sandborn, 1994). The remaining 61% of the

pouchitis patients may experience more than one episode of inflammation. Only patients with chronic or continuous pouchitis have major morbidity. This group consists of 15% of all pouchitis patients. Pouch failures and excisions in these circumstances are up to 40% of these patients, which translates to a total of 2% excision rate due to pouchitis out of all pouch cases (Sandborn, 1994).

There is a hidden danger of dysplasia in some of the pouches that requires surveillance for the future. It is possible that a few of these patients will require excision eventually; the follow up period extends to the end of the second decade after the introduction of this type of surgery (Sandborn, 1994).

## **2.B. INFLAMMATORY BOWEL DISEASE**

### **2.B.1. Inflammatory Bowel Disease Assessment**

#### **2.B.1.1. Introduction**

The two main forms of non-specific inflammatory bowel disease recognised are Crohn's disease and ulcerative colitis. Crohn's disease can affect any part of the gastrointestinal tract, while UC affects only the large bowel (Kumar 1994). Hence the attraction of the surgical treatment in UC, which offers permanent cure to the patient. Furthermore, with the creation of an ileoanal pouch, this cure is achieved avoiding a permanent ileostomy, which has an obvious psychological advantage.

It has been recognised however, that there is certain overlap between CD and UC not only in the clinical features but also in the histological characteristics and radiological findings. Approximately 10% of the colitis cases cannot be categorised with certainty to one or the other of the inflammatory bowel diseases. Accordingly they are referred to as indeterminate colitis (IC), until the actual natural history of the disease manifests the true underlying pathology (Kumar, 1994).



The clinical presentation in CD includes diarrhoea, abdominal pain and weight loss; in Crohn's colitis the diarrhoea may be bloody. In UC diarrhoea is often bloody with mucous, while the abdominal pain is more like discomfort with tenesmus. Both inflammatory bowel diseases may be accompanied by constitutional symptoms of malaise lethargy and anorexia, but Crohn's patients may also complain of nausea, vomiting and low grade fever. Extraintestinal manifestations may be present in either of the two diseases. In CD the development of complications like stricture, abscess and fistula formation, may pose real management dilemmas. Often it is difficult to identify, whether a particular symptom is due to the disease process of gut inflammation or to the emergence of these complications. Furthermore, medical treatment may change the presentation of the disease by introducing its own side effects. Nutritional, social and psychological factors further add to the puzzle of the disease morbidity and complex presentation (Kumar, 1994).

### **2.B.1.2. Disease Activity Indexes In IBD**

The accurate assessment of the therapeutic manipulations made necessary the introduction of methods destined to measure the degree of inflammation associated with the disease, so meaningful comparisons between the various therapeutic options may be possible. It was evident, however that patients who had clinically inactive disease may have features of inflammation in their gut, as measured by granulocyte excretion (Saverymuttu 1986). Alternatively, systemic indices of inflammation like the erythrocyte sedimentation rate and serum C-reactive protein concentration do not always reflect the gut related immune processes.

In UC, Truelove and Witts devised a simple classification of the severity of colitis: severe disease was defined as the passage of six or more stools per day with blood, accompanied by fever, tachycardia, anaemia, or rapid ESR (Keighley 1993a).

Another useful index for assessment of disease activity in UC was devised by Powell-Tuck during a prospective study to compare two different regimes of steroid treatment (Powell-Tuck et al, 1978). This index includes parameters like

general health (score 0-3), abdominal pain (score 0-2), bowel frequency (score 1 or 2), stool consistency (score 0-2), blood in stool (score 0-2), anorexia, nausea, abdominal tenderness (score 0-3), eye inflammation, arthralgia, oral ulceration, related skin lesions (score 0-2), temperature (score 0-2), and sigmoidoscopy (score 0-2). It is obvious that this index has many similarities with Crohn's Disease Activity Index (CDAI).

The CDAI was devised by gastroenterologists from 13 institutions as a method to monitor the progress of CD after collecting data from 187 patient visits (Best et al, 1976). The dependent variable used for each patient visit was the physician's over-all evaluation of "how the patient was doing". This variable was then related to 18 independent variables, which could be readily available at each visit. With a multiple regression with stepwise deletion, 8 variables were identified as independent parameters. Each of them was rounded in such a way to give simple figures for calculation of the CDAI. In practice, the patient records in a card, for seven days prior to his/her consultation, the number of liquid or very soft stool, the presence of abdominal pain, and his general well being. The rest of the parameters are filled in by the physician: presence or absence of six complications (arthritis, iritis, skin complications, anal fissure, fistulae, fever), use of anti diarrhoeal medication, presence of abdominal mass, value of haematocrit and the body weight. A value of CDAI above 150, was agreed to signify active disease.

Over the years other disease activity indexes were improvised in an attempt for simplicity and to minimise the bias of the subjective symptoms incorporated in the CDAI. Notable examples are the OMGE disease index from the World Organisation of Gastroenterology and the International Organisation for the study of Inflammatory Bowel Disease (IOIBD). Harvey and Bradshaw (1980) created a CDAI modification that is widely used in Britain (Keighley, 1993b).

An endoscopic index was also devised which included the severity of ulceration, extent of disease, presence of stenosis with or without ulceration (Mary & Modigliani, 1989). Furthermore, a whole range of parameters was proposed: erythrocyte sedimentation rate, the C-reactive protein, orosomucoid,

faecal  $\alpha_1$ - antitrypsin or its clearance, faecal  $^{111}\text{Indium}$  excretion. More recently, other parameters were described: assessment of microalbuminuria (Mahmud et al, 1996), DNA flowcytometric evaluation of cell cycle distribution of rectal biopsy specimen for UC (Bortoluzzi et al, 1995), Doppler sonography of superior mesenteric artery flow (Van Oostayen et al, 1997), serum thrombomodulin in UC (Boehme et al, 1997), leukocyte adhesiveness/aggregation test (Arber et al, 1995), urine levels of soluble TNF receptors p55 and p75 (Hadziselimovic et al, 1995).

The question, however that emerges is what is the biological phenomenon, if any, that CDAI and the other similar clinical activity indexes measure. These indices are certainly a reflection of the overall clinical impression of the physician of "how well the patient is doing". De Dombal though, made a disquieting point that there is considerable interobserver variation in calculating the various indices used for the disease activity between clinicians. (De Dombal & Softtley, 1987).

Saverymuttu originally found a high correlation of the faecal excretion of  $^{111}\text{Indium}$  labelled leukocytes and the CDAI ( $r=0.78$ ,  $p<0.001$ ; Saverymuttu et al, 1983). In later studies, however, he admitted that patients in clinical remission as judged by a CDAI less than 150, may have significantly higher excretion of faecal  $^{111}\text{Indium}$  compared to control group of irritable bowel syndrome (Saverymuttu, 1986). Instead the endoscopic and histological scores correlated well with the  $^{111}\text{Indium}$  scans (Saverymuttu et al, 1986). Similar results were observed with the use of the  $^{99m}\text{Tc}$ -HMPAO labelled autologous granulocytes (Sciarretta et al, 1993).

The relations between clinical activity, endoscopic severity and biological parameters in CD were assessed in 121 consecutive patients with colonic or ileocolonic disease. Stepwise linear regression identified the C-reactive protein as predictive of the clinical index (CDAI), and, successively  $\alpha_2$ - globulin, erythrocyte sedimentation rate, faecal  $\alpha_1$ -antitrypsin and serum orosomucoid as predictive of the endoscopic index. However, both predictions were poor, indicating that clinical activity in CD is virtually independent of the severity of the mucosal lesions and biological activity (Cellier et al, 1994).

Protein losing enteropathy is a measure of extent or severity of CD (Chadwick & Camilleri, 1990); recently this was substantiated in a prospective study using  $^{51}\text{CrCl}_3$  labelled proteins and measuring their faecal excretion (Nordgren et al, 1990). Studies also with the whole gut lavage fluid methodology have shown that the CDAI is closely related to this protein losing enteropathy (see following section 2.B.1.3).

The Pouchitis Disease Activity Index (PDAI) is another example of the creation of an index in an attempt to objectively measure disease activity in a unique inflammatory disease process of the gut i.e. pouchitis. It is obvious that it also has a heavy bias from the stool frequency and urgency, which may be due to other pouch pathologies. The endoscopic and histopathological aspect of the PDAI may be biased from sampling error. It is not influenced by haematocrit; it does not take into account anti-diarrhoeal medication; and it ignores largely the extraintestinal manifestations.

### **2.B.1.3. Whole Gut Lavage Fluid - Assessment Of Disease Activity In IBD**

The limitations of all these indexes were a trigger for intense research in an attempt to identify a practical and objective method to assess disease activity in inflammatory bowel disease that will be clinically relevant, reproducible and well tolerated. This work led to the Whole Gut Lavage Fluid (WGLF) methodology. In this section, I will introduce the way the WGLF methodology was developed in our Gastrointestinal Laboratory and explain how it was used to assess various aspects of inflammation, injury and bleeding in the gut.

Whole gut lavage with a polyethylene glycol electrolyte solution is extensively used in clinical practice for bowel preparation prior to surgery or colonoscopy. It has also been utilised as a form of treatment for various poisoning states (Jawary et al, 1992). It is well tolerated. Only one serious adverse event has been reported in relation to the WGLF method; a child developed acute pulmonary oedema during a whole gut irrigation via a nasogastric tube. From the description however, it is not entirely convincing that the

pulmonary oedema was not due to aspiration of the stomach contents (Paap & Ehrich, 1993).

Gaspari et al, (1988) described a method of utilising the gut lavage to detect immunoglobulins in 12 human volunteers. The main problem they faced was that the human intestinal secretions contained an abundance of proteases. With the addition of various protease inhibitors this problem was overcome.

O'Mahony et al, (1990) showed that lavage specimens which were faecally contaminated contained negligible quantities of immunoglobulins. On the other hand, once the specimens became clear a steady state was reached, with little variation in immunoglobulin content between serial specimens and with a uniform dilution (around 20%) of the ingested polyethylene glycol (O'Mahony et al, 1990). ELISA techniques were used in the measurements of total IgA, IgM and IgG in WGLF in healthy controls, in coeliac disease and in CD patients. A striking difference noticed was that the IgG content of the WGLF of patients with active CD was at least 10 times higher than the concentration in the normal group (O'Mahony et al, 1990).

Prompted by this finding, another study was conducted where the IgG in the WGLF of a larger group of IBD patients with active or inactive disease was compared with controls. "Disease activity" was assessed by an independent clinician on review of current symptoms and signs; radiological, endoscopic and histopathological features; haematological and biochemical indices: ESR, platelet count, C-reactive protein, plasma protein, and albumin. This time, it was noticed that active IBD was characterised by significantly higher levels of IgG both compared to inactive disease and the controls (O'Mahony et al, 1991). Furthermore, the albumin measured in the WGLF of these patients seemed to mirror the IgG levels ( $r = 0.68$ ,  $p < 0.0001$ ). This finding signified that the IgG measured in the WGLF was probably plasma derived, in spite of the known phenomenon of high levels of IgG secretory plasma cells in the lamina propria in IBD patients (O'Mahony et al, 1991).

It was evident, from the above studies, that disease activity in IBD may be associated with protein loss as judged by the IgG content of the WGLF. A prospective study of WGLF parameters was undertaken, where the disease

activity was measured along with the CDAI or the Powel-Tuck (PTI) indices for CD or UC respectively. During this study another protein,  $\alpha$ -1-antitrypsin (A1AT), was also assessed. It was shown that IgG in CD strongly correlated with the CDAI ( $r=0.723$ ,  $p<0.0001$ ). A similar observation was true for IgG and the PTI in UC ( $r=0.714$ ,  $p<0.0001$ ). The results for albumin and A1AT were similar to those of IgG, but were less sensitive in detecting active disease (Choudari et al, 1993). It was argued in this paper, that if the resultant increase of concentration of the above proteins in the WGLF was merely due to bleeding, then their concentration should mirror their plasma concentrations. This in fact is not the case. It was also shown that albumin in unprocessed WGLF degrades more rapidly than the other proteins, with a reduction in measured quantity of 10-40% in one hour (Choudari et al, 1993). This study, therefore demonstrated that measuring WGLF IgG was a new objective and accurate method to quantify disease activity in IBD; it also showed that the disease activity as described by the CDAI and the PTI is analogous to the gut protein loss that happens in inflammation of the bowel.

Ground work on the validity of the method was performed; polyethylene glycol, residual protease activity, IgA, IgM, IgG, albumin, A1AT and titres of IgA antibody to *Salmonella typhi* LPS were similar in five sequentially collected WGLF specimens (Ferguson et al, 1995). This was also an indication that the system established when the patient was drinking the polyethylene glycol and at the point of the production of the clear specimen was at a steady state i.e. the fluid obtained was a gut perfusate. Sixty-three immunologically normal patients and 254 with various gastrointestinal symptoms were tested and reference levels were established for WGLF IgG, albumin, and A1AT. The normal ranges given were 1-10  $\mu\text{g/ml}$  for IgG; 1-26  $\mu\text{g/ml}$  for albumin; 1-19  $\mu\text{g/ml}$  for A1AT (Brydon et al, 1993). The gut protein loss as defined by the WGLF was consistent, in the majority of patients, with the clinical picture (one lymphangiectasia, seven colorectal cancers, one gut lymphoma, one perforated diverticulitis, one pouchitis and one lymphocytic colitis). The level of IgG in WGLF, when used in IBD patients, could accurately define active and inactive disease, in a similar way as in the prospective study mentioned above.



#### **2.B.1.4. Gastrointestinal Blood Loss In WGLF**

Another area of investigation was occult gastrointestinal bleeding. To quantify the severity of this phenomenon, WGLF haemoglobin was examined. This was found to be higher in patients with colorectal cancer, severe diverticular disease and rectal varices. Seven out of sixteen patients with IBD and four patients with iron deficiency anaemia of gastrointestinal origin had raised WGLF haemoglobin (Brydon & Ferguson, 1992). In parallel to that, subjects with healthy gastrointestinal tracts had WGLF haemoglobin concentration measured: range of 0.5-5.1 µg/ml. A comprehensive analysis of iron deficiency anaemia referrals, with clinical, endoscopic and WGLF haemoglobin methodology, showed that occult gastrointestinal bleeding sufficient to cause anaemia was evident in only 19% of the cases, the rest having a plethora of other potential causes for their iron deficiency, suggesting that most of the gastrointestinal lesions detected in them were probably coincidental (Ferguson et al, 1996).

#### **2.B.1.5. Pro-Inflammatory Cytokines In WGLF**

Lymphocytes and macrophages synthesise and secrete a number of potent pro-inflammatory mediators. Interleukins IL-1 $\alpha$  and IL-1 $\beta$  are of particular interest, since human T-cell activation is promoted by IL-1 presence. IL-1 also promotes B-cell activation, while the best known IL-1 activities are fever and stimulation of acute phase protein synthesis. In IBD, Mahida et al (1989) studied IL-1 $\beta$  release from isolated intestinal lamina propria mononuclear phagocytes and observed enhanced spontaneous and LPS induced secretion from monocytes. Others described raised levels of IL-1 $\beta$  in mucosa of endoscopic biopsies, correlating with disease activity (Brynskov et al, 1992).

Other cytokines, including TNF $\alpha$  and IL-6 have also been studied. These were associated with the immune process in IBD (Isaacs et al, 1992). The interleukin IL-8 is a potent chemoattractor and activator of neutrophils, which can cause much of the mucosal destruction during migration from the endothelium

into the gut lumen. Raab et al (1993) measured IL-8 in rectal perfusates in IBD patients and found that levels of this cytokine correlated well with the myeloperoxidase activity that was simultaneously assessed.

The work performed on the cytokine profile in WGLF in various entities was of considerable interest. Bioassays were found to be not suitable for the WGLF, but instead enzyme linked immunosorbent assays (ELISA) and radio immunoassay (RIA) techniques could readily be applied. Whole gut lavage was performed in a mixed group of patients who received elemental diet EO28. WGLF was obtained before and after elemental diet. The clinical responders to this form of treatment manifested a reduction of the WGLF levels of soluble IL-2 receptor, which is a marker of T-cell activation. In these patients however, the serum sIL-2R did not change (Ferguson et al, 1994). Studies on TNF in IBD, measured in WGLF, showed that TNF was detectable in both CD and UC. This correlated more closely to disease distribution (colonic) than to disease activity (Ferguson & Mwantembe, 1995). Studies on IL-1 $\beta$  and IL-8 of the WGLF showed that these proinflammatory cytokines are higher in active IBD (Ghosh, 1995). Cytokine work in cystic fibrosis children showed that a subgroup with distal intestinal obstruction syndrome treated with Nutrizym 22, had a very abnormal cytokine profile in their WGLF, with an added elevation of the WGLF albumin (Croft et al, 1995).

#### **2.B.1.6. Neutrophil Migration In IBD & The WGLF**

Neutrophil polymorphs are a prominent feature of the intestinal inflammatory infiltrate in IBD. Amplification of this neutrophil response is largely due to activated phagocytes in situ releasing the potent neutrophil chemoattractant leucotriene B<sub>4</sub> (Lobos et al, 1987).

Several studies over the years have been described to assess the chemotaxis of neutrophils in IBD. These are presented in the next section (see section 2.B.2). *In vivo*, Saverymuttu showed that peripheral blood leukocytes, labelled with <sup>111</sup>Indium, injected in IBD patients quickly accumulate in the inflamed intestine (Saverymuttu et al, 1985a; 1985b). Furthermore, these leukocytes within a few days appear in the faeces. Accordingly a 4-day faecal



collection for measurement of  $^{111}\text{In}$  excretion was introduced. This combined method of labelled leukocyte scintigraphy and faecal collection was used in clinical practice for assessment of the disease extent, the disease activity, for screening for IBD and the detection of complications of IBD (Giaffer 1996).

Another radioisotope was later used, Technetium-99m hexamethyl propylene amine oxime ( $^{99\text{m}}\text{Tc HMPAO}$ ). The advantage of this scan is that it gives superior quality images, the radioisotope is readily available and the technique does not require the time consuming granulocyte separation. The radiation dose involved is low. The only disadvantage is that it cannot be used for faecal excretion studies since it has a short half life and has a biliary excretion (Giaffer, 1996). The introduction of the  $^{99\text{m}}\text{TC-HMPAO}$  labelling along with the single photon emission computerised tomography (SPECT) allowed visualisation of the entire bowel separate from overlying structures (Weldon et al, 1996).

It was evident, from these scintigraphic studies, that there is a phenomenon of neutrophil migration to the gut lumen in IBD. It is feasible to hypothesise that these "luminal" neutrophils that have migrated through endothelium and epithelium, have characteristics very similar to the exudative neutrophils: enhanced microbicidal activity, expression of CD11b, CD16 and the FMLP receptor (Yee et al, 1994).

Animal studies on FMLP, a potent granulocyte activator that is produced by bacteria resident in the colon and ileum, have shown that mucosal permeability is increased by FMLP in the distal 10 cm of the terminal ileum. Furthermore, this increase is prevented by rendering the animal neutropenic. Activated neutrophils release both oxidants and proteases; the latter can be rapidly inactivated by antiproteases normally found in extracellular fluid (e.g.  $\alpha 1$ -antitrypsin). However, neutrophil derived oxidants are believed to alter the protease/antiprotease balance by inactivating antiproteases. Thus, neutrophil derived oxidants and proteases work in concert to mediate the increase in mucosal permeability elicited by FMLP (Crissinger et al, 1990).

Two observations relevant to the issue of the importance of luminal neutrophilia is the observation of an enterohepatic circulation for FMLP in experimental colitis in rats (Hobson et al, 1988) and of immunoreactive bacterial

chemotactic peptides in man (Roberts et al, 1990). Furthermore, increased neutrophil receptors for FMLP were discovered in patients with CD (Anton et al, 1989). It is noticeable that oxidised and reduced forms of FMLP can be detected in bulk environmental organic dust samples (Siegel et al, 1994).

Cytology of the WGLF was performed in an attempt to investigate neutrophil migration into the gut lumen (Handy et al, 1995). It was shown that luminal neutrophilia characterised active UC and active colonic CD, but not active small bowel CD, or inactive disease. This was in agreement with earlier work on faecal Indium<sup>111</sup> excretion experiments (Crama-Bohbouth et al, 1989). This work was expanded to the utilisation of neutrophil granulocyte elastase measurement in WGLF, with similar results, since the cytology methodology proved to be rather laborious (Handy et al, 1996).

#### **2.B.1.6. Other Uses Of WGLF Methodology In IBD**

The method has now been established as acceptable for routine clinical practice in the objective assessment of IBD disease activity and blood loss. The research took on various directions. Levels of IgA antibodies to *bacteroides fragilis* LPS were measured in CD and found to be higher (Poxton et al, 1995). The total IgA in WGLF was also assessed in smokers as compared to UC patients and found to be lower (Srivastava et al, 1991). The WGLF IgA antibodies to *Klebsiella pneumoniae* were studied in normal controls, patients with CD and patients with ankylosing spondylitis and found to be similar in spite of the apparent differences in serum IgA between the normal group and the other two groups with high IgA (O'Mahony et al, 1992).

Other substances have been measured in WGLF, which although they appear to have no direct impact on the immediate research in IBD, may in the future enlighten aspects of the pathological mechanisms involved. Ghosh showed that Insulin-like growth factor-1 and TGF- $\beta$  in WGLF can be utilised as markers of intestinal fibrosis (Ghosh et al, 1997). Measurement of the cytokine RANTES or of the eosinophil cationic protein can assess eosinophil activity in various pathologies in the gut (Handy et al, 1995).

The advantages of adopting the WGLF methodology to study the immunity of the gut in IBD are clearly considerable. This can be reinforced by two more observations. First observation; when faecal extracts of immunoglobulins, albumin, A1AT and isotype specific antibodies were compared with the estimated daily output (the amount known to be produced from the gut with WGLF assays) the recovery rate was extremely variable (Ferguson et al, 1995). This finding demonstrated that probably saline faecal extracts do not represent the true status of the gut humoral system and that they better be looked upon with caution. Second observation; when the CDAI was compared with various clinical subgroups of patients, some discrepancies could be identified. Firstly patients with clinically inactive IBD, but with fibrous small bowel strictures would have high CDAI score but a normal IgG in the WGLF. This was due to the overemphasis given by the CDAI to the clinical aspects of disease. Consequently the CDAI was also raised in psychologically disturbed patients, for whom the Clinicians' assessment was of inactive disease. In these cases the WGLF IgG was again low. Thirdly in children, the CDAI had no relation whatsoever to the activity of the disease as assessed by the Clinician. The WGLF IgG, however could fairly accurately characterise them. Fourthly, the CDAI in ileostomy patients did not correlate with any of the parameters studied. The IgG though, was again responding very well to the global clinical assessment (Acciuffi et al, 1996).

## **2.B.2. Neutrophil Chemotaxis**

### **2.B.2.1. Introduction**

The interest in chemotaxis (directional movement) or chemokinesis (random movement) of leukocytes in IBD has been based on the assumption that neutrophils are one of the main components in acute inflammation. Thus, their excessive aggregation in IBD gut mucosa should follow a possible abnormality in their function.

One of the most influential studies in this field was the work of Segal and Loewi (1976). They observed that leukocytes from CD patients exhibited decreased chemotaxis to their own sera in skin window chambers. This may be explained, either by defective chemotaxis of neutrophils or by the appearance of anti-chemotactic agents into the sera. Many other investigators have reproduced this finding and attempted to explain its occurrence. Since the methodology used by individual investigators differed both in the particular assays used and in the various chemoattractants utilised, a considerable variation of outcome and resulting opinion has arisen.

### **2.B.2.2. Chemotaxis In IBD**

Neutrophils from UC patients showed decreased chemotaxis to casein *in vitro*, while their spontaneous motility, or chemokinesis, in active disease was enhanced (Binder & Riis, 1977). One UC patient was reported, who had abnormal chemotaxis, thought to stem from the presence of a serum inhibitor in the form of IgA (Patrone et al, 1978). Corticosteroids and sulphasalazine reduced chemotaxis, a phenomenon which was irrespective to response to treatment (Hermanowicz et al, 1985; Rhodes et al, 1981). Other investigators however found no difference *in vitro* chemotaxis whether spontaneous or casein stimulated in Boyden chambers in either UC or CD (Wandall, 1985). This finding was supported also by Elmgreen, who again did not find any difference in chemotaxis, when the chemoattractant was casein. Instead he observed reduced chemotaxis to the complement fraction C5a both in UC and CD patients, who had no treatment for at least three months (Elmgreen, 1984). In another study in CD, he showed that the *in vitro* activation of the complement cascade produced in the alternative pathway a defective C5a with under utilisation of C3 (Elmgreen et al, 1983). On the other hand, a reduction of chemotaxis was found in active disease in UC when compared with inactive UC. This was not accompanied by changes in motility of neutrophils at the skin window chamber experiments (Elmgreen & Binder, 1982).

The skin window chamber chemotaxis is reduced in CD, but in the *in vitro* experiment no differences were noted. Furthermore, the patients' sera could not modify the chemotaxis effect (Morain et al, 1981). Rhodes and Jewell however,

demonstrated the presence of a substance in serum of active CD patients, which was similar to the 7S  $\gamma$ -globulin, and could be precipitated by the 40% ammonium sulphate. This substance was a serum inhibitor of chemotaxis (Rhodes et al, 1982).

In another study, Rhodes and Jewell found no decrease *in vitro* chemotaxis or chemokinesis in either UC or CD, as far as the neutrophils were concerned, but noticed a slight increase of the monocyte chemotaxis in UC (Rhodes & Jewell, 1983). Other investigators found similar increase of monocyte chemotaxis in UC, but this did not show statistical significance. On the other hand, increased chemokinesis was demonstrated in both UC and CD cases (Whorwell et al, 1981).

When the agarose method for chemotaxis was employed, there was a reduction in chemotaxis, *in vitro*, which on this occasion was related to disease activity (Belsheim et al, 1984). Others observed that, whereas all UC patients had originally low chemotaxis compared to the normal controls, when these patients were studied during the active phase of their disease their corresponding chemotaxis increased (Hermanowicz & Nawarska, 1981). The opposite was observed in CD patients. Active CD presented lower chemotaxis and inactive disease higher (Belsheim et al, 1981). Lennard-Jones team showed similar reduction in chemotaxis in UC and CD; but in active UC the immunofluorescence after incubation of normal neutrophils and sera from UC was analogous to the impaired cell migration (Kirk et al, 1983).

These discrepancies in the results may be due to the different methodology and assays used by each investigator. However, Wandall managed to clarify some of these problems by introducing an extra parameter, which was missing in the previous presentations, i.e. the time scale of the biological phenomena observed. The skin window chamber test performed in UC showed no difference at the first 12 hours, but 24 and 48 hours later revealed lower total number of neutrophils present and lesser rate of migration (Wandall & Binder, 1982). This mobilisation was dependent on the total count of neutrophils in the blood of the patient. The *in vitro* chemotaxis was reduced. In CD, there was a lag phase initially and then

again the migration was reduced, while the chemotactic response to casein was not affected (Wandall & Binder, 1982).

In the following years the interest moved towards the importance of arachidonic acid derivatives and their effects in chemotaxis.

Serum from UC patients, who have been given fish oil supplements (MaxEPA) was found to be less chemotactic, while the neutrophil LTB<sub>4</sub> content was reduced at the same time (McCall et al, 1989). Nielsen experimented in the Boyden chamber with neutrophils from UC and CD patients compared to controls using different concentration of casein, FMLP, C5a, LTB<sub>4</sub> and its metabolites, and 5-HETE and its metabolites. The chemotaxis was reduced in both diseases at the suboptimal concentrations of LTB<sub>4</sub> and 5-HETE, while the peak response of casein, FMLP, LTB<sub>4</sub> and 5-HETE of IBD neutrophils were similar to normals. The peak response to C5a was reduced in IBD. The LTB<sub>4</sub> appeared to be 100 times more potent chemotactic factor than 5-HETE (Nielsen & Elmgreen, 1987). These findings with the FMLP chemotaxis were not observed on filter assays in neutrophils from CD patients, whereas in other series, these neutrophils from CD patients were found to exhibit increased chemotaxis. Similar increase in chemotaxis was also seen for those neutrophils when *Candida albicans* was used as the chemoattractant (Curran & Keighley, 1991).

An observation was made in two patients with Crohn's like colitis and glycogen storage disease Ib. These patients had decreased neutrophil chemotaxis *in vitro* to FMLP, zymosan-activated serum and *Escherichia coli* bacteria derived factor. This raised the hypothesis, whether the chemotaxis abnormality was the linking factor between the colitis and the glycogen storage disease (Couper et al, 1991).

In a large scale experiment, Hermanowicz compared *in vitro* neutrophil chemotaxis between controls and UC patients using either complement derivatives or a mononuclear derived chemotactic factor (MDCF). The chemotaxis to complement derivatives was again defective, whereas no problem was observed with the MDCF (Hermanowicz & Nawarska, 1986).



### **2.B.2.3. Chemotaxis In IBD & The Bowel Wall**

The main problem with all these studies is that they address chemotaxis in neutrophils as part of the systemic immune phenomenon and do not consider the interactions of the neutrophils and the gut mucosa as a whole.

Lobos et al (1987) studied colonic biopsies from 3 normal controls and 9 IBD patients after they had been homogenised and showed an increase in chemotaxis of human neutrophils for the IBD samples in a Boyden chamber. Further analysis of the homogenates showed that chemotaxis was mostly due to a lipid extractable substance, which with the help of reverse phase high pressure liquid chromatography could be coeluted with LTB<sub>4</sub>. The amount of chemotactic activity correlated well with the concentration of leucotriene B<sub>4</sub> measured by UV absorbance.

Kazi et al (1995), exposed normal neutrophils to rectal dialysates from control subjects, and from patients with active and inactive UC. He then measured reactive Oxygen species (ROS) production with chemiluminescence. He discovered that active UC dialysates significantly increased the production of ROS. When he stimulated the neutrophils with either FMLP or phorbol myristate acetate (PMA), dialysates from the control group would mostly inhibit this stimulation. In contrast, 44% of rectal dialysates from active UC could actually potentiate the effect of FMLP, but only 13% in the control samples could do so.

It is possible that the neutrophil as it passes through the mucosa to reach the lumen requires the influence of separate mechanisms at each stage. This hypothesis may be substantiated by the following observations. The first is that neutrophils in crypt abscesses in IBD seem to contain ELAM-1. At the same time ELAM-1 is mainly seen at the areas of the mucosa where inflammation was present, while VCAM-1 was uniformly distributed (Koizumi et al, 1992). The second observation is that bleeding (tissue injury as judged by faecal excretion of chromium-51 labelled red cells) correlates with intestinal inflammation (measured simultaneously by faecal excretion of <sup>111</sup>Indium labelled leukocytes)

in UC and not in CD. This may indicate that the possible chemoattractant in UC is luminal whereas in CD is mucosal (Teahon & Bjarnason, 1993).





# **CHAPTER III**

## **MATERIALS & METHODS**

**A. PATIENTS**

**B. METHODS**

## **3.A. PATIENTS**

### **3.A.1. Introduction**

This chapter describes the patients recruited for the study and the methodology followed. This includes patients who underwent restorative proctocolectomy for ulcerative colitis, indeterminate colitis, familial adenomatous polyposis coli and other reasons which will be discussed in the following sections. Patients characteristics are described. Details of patients who underwent restorative proctocolectomy within six months of the study will be given in a separate group 'early group'. The patients and methodology of the ileostomy patients recruited will be presented at the end of this chapter.

### **3.A.2. Patient Recruitment**

Seventy-four consecutive patients who were attending the General Surgical Department in the Western General (12 patients) and the Royal Infirmary of Edinburgh (62 patients) between October 1994 and March 1996 were recruited for the study. They all had previous restorative proctocolectomy.

Five patients were excluded from the study: 1 patient was not able to attend due to employment difficulties; 1 patient did not participate due to travelling long distance to Edinburgh; and 3 simply refused to participate in the study.

Seventy patients out of the 74 recruited were attending for follow up in the Surgical Outpatient Clinic. Four, were inpatients: 1 in the Western General Hospital and 3 in the Edinburgh Royal Infirmary.

From the 74 patients recruited, 3 had the procedure repeated twice. These were patients with 'pouchitis' on different treatment regimes each time. Only the 1st patient-episode was used in our later analysis of results.

Three patients did not have the study completed according to the protocol. They were excluded from further analysis. As it will be explained in detail at the next section (see section 3.B), the protocol included the whole gut lavage, the

patient questionnaire, pouchoscopy and biopsy. Biopsy was omitted in 1 patient because of the high prothrombin time and the risk of bleeding, and in 2 other patients due to patients' intolerance. Therefore, 71 patients' results were finally included in the analysis.

Between 1/1/1991-31/12/95, from the data available for the Royal Infirmary, 95 patients had restorative proctocolectomy under Mr DCC Bartolo. The period of the study, in which recruitment took place for the Royal Infirmary, was February 1995 till February 1996. From this cohort of 95 patients, 62 consecutive patients eventually did have the whole gut lavage study (65%). It was not possible to recruit 35% of patients. The reason for this was that their outpatient appointments were outwith the study period; alternatively a number of patients resided outside the Lothian Region and opted for follow up in their local Hospital.

Between 1/1/1991-31/12/95, from the data available for the Western General Hospital, 22 patients had restorative proctocolectomy under Mr DW Hamer-Hodges. The period of study in which recruitment took place for the Western General Hospital was February 1995 till February 1996. February 1995 till February 1996 From this cohort of 22 patients, 12 consecutive patients eventually did have the whole gut lavage study (55%). It was not possible to recruit 45% of patients. The reason for this was that their outpatient appointments were outwith the study period; alternatively a number of patients resided outside the Lothian Region and opted for follow up in their local Hospital.

### **3.A.3. Patients' Characteristics**

Patients' age ranged from 18.2 to 76 years (median: 41.7 years). The male to female ratio was 40:31. The period of time between the study and the creation of pouch ranged from 1.1 to 83.7 months postoperatively (median: 26.9; 95%CI: 22.8, 31.1 months). For those patients who had a covering ileostomy, this time was calculated from the time they had their ileostomy closed.

### **3.A.4. The Patient Groups**

#### **3.A.4.1. 'Ulcerative Colitis Group' & 'Early Group'**

Sixty-one patients had restorative proctocolectomy for Ulcerative Colitis (UC). The clinical characteristics of the UC patients will be analysed in detail in chapter IV. The male to female ratio was 36:25. Patients' age ranged from 18.2 to 74.6 years (median: 41.9 years). The period of time between the study and the creation of pouch ranged from 1.1 to 83.7 months postoperatively (median: 26.6 months; 95%CI: 22.5, 32.0).

Analysis of the data of these patients showed that patients who had pouch constructed less than six months from the day of the study, had some distinct clinical characteristics (see chapter IV). This may be related to the fact that these patients underwent major surgery in the non-distant past from the study. Furthermore, the prospective histological study from Apel et al (1994) indicated that a period of six months is required for the histological changes in the pouch mucosa to stabilise. Consequently, a subgroup of UC patients, called 'early group' in our study, was formed and analysed separately. Five patients constituted this group (see chapter IV). The male to female ratio was 4:1. Patients' age ranged from 23 to 35 years (median: 36.3 years). The period of time between the study and the creation of pouch ranged from 1.0 to 5.8 months postoperatively (median: 3.4 months).

The remaining fifty-six patients, who had pouch constructed in greater than six months from the date of the study, constituted the 'Ulcerative Colitis group'. This group concluded the main body of our study (see chapter IV). The male to female ratio was 32:24. Patients' age ranged from 18.2 to 74.6 years (median: 42.8 years). The period of time between the study and the creation of pouch ranged from 6.5 to 83.7 months postoperatively (median: 28.9; 95%CI: 24.4, 34.7).

#### **3.A.4.2. 'Indeterminate Colitis Group'**

Indeterminate colitis (IC) patients may have some different clinical characteristics concerning their pouch (see chapter II; McIntyre et al, 1995;

Koltun et al, 1994). It was therefore, considered appropriate to study this cohort of patients separately from the main body of UC patients. Five of 71 patients had their pouch constructed for what it could have been characterised as IC in the colectomy specimen. Four of them had emergency surgery: 3 for toxic megacolon and 1 for diverticular abscess. One of the patients with toxic megacolon developed diversion colitis in the defunctioned rectum, before the pouch construction. Only 1 patient had routine surgery, restoration and colectomy done as one procedure. No patient has been operated for Crohn's Colitis.

In this group, the male to female ratio was 1:4. Patients' age ranged from 35.7 to 61.0 years (median: 43.7 years). The period of time between the study and the creation of pouch ranged from 7.4 to 39.0 months postoperatively (median: 26.5 months).

### **3.A.4.3. 'Non Inflammatory Bowel Disease Group'**

Five of 71 patients had their pouch constructed for reasons other than UC: 2 patients had pouch for multiple synchronous cancers of the colon; 1 patient had the operation for Familial Adenomatous Polyposis Coli; 1 patient had restorative proctocolectomy for intractable constipation with previously failed ileorectal anastomosis. The male to female ratio was 3:2. Patients' age ranged from 23.2 to 76.8 years (median: 48.2 years). The period of time between the study and the creation of pouch ranged from 7.5 to 46.0 months postoperatively (median: 25.6 months).

## **3.B. METHODS**

### **3.B.1. Introduction**

The methods included tests already well established in the investigation of ileoanal pouches and tests well described and established for the evaluation of bowel disorders, i.e. the Whole Gut Lavage methodology. In the presentation of

our methodology, the whole gut lavage process will first be described and then the clinical procedures followed will be examined. The study of chemotactic potential of the whole gut lavage fluid will be presented in chapter VIII.

### **3.B.2. 'Pilot' Study**

To check the feasibility of the project, the medical and laboratory notes of 8 patients who previously had a whole gut lavage study of their pouch were examined. During this retrospective study of the notes, the clinical problem of each patient was considered in association with the Whole gut lavage results. From these preliminary observations, it was evident that there were differences in the whole gut lavage parameters that could be used to distinguish between the various diagnostic groups.

### **3.B.3. Whole Gut Lavage**

#### **3.B.3.1. Introduction**

Gut lavage with a polyethylene glycol (PEG) electrolyte solution has been extensively used in clinical practice for bowel preparation prior to surgery, colonoscopy and barium enema. When this particular methodology of performing the gut lavage is adhered to, then the resultant whole gut lavage fluid (WGLF) becomes a true gut perfusate (Ferguson et al, 1995). This is readily acceptable for use in the study of mucosal immune events.

#### **3.B.3.2. Formulation Of Lavage**

The polyethylene lavage electrolyte solution, which was utilised, was constituted by dissolving one sachet of Klean- Prep® (Forgone Ltd, Harefield, Middx, UK) in 1 litre of tap water. Each sachet contained 59.00g of polyethylene glycol 3350 USNF. PEG 3350 is a mixture of different sized molecules with a mean molecular weight between 3200 and 3700. Other ingredients per sachet of

Klean- Prep® include 5.685g of sodium sulphate anhydrous Ph Eur; 1.685g of sodium bicarbonate Ph Eur; 1.465g of sodium chloride Ph Eur; 0.743g of potassium chloride Ph Eur; 0.0494g of aspartame USNF.

The resultant solution can be used, if refrigerated, for up to 24 hours. The initial content of fluid of 59 g/L PEG will reduce to 47.5 g/L at the end of the processing phase.

### **3.B.3.3. The Protocol Of Whole Gut Lavage**

The patient fasts overnight, and then consumes the PEG solution at a steady rate. The rate of consumption is kept to 1 litre per hour adhering to the protocol, supervised by an experienced research nurse (Choudari et al, 1993).

Nausea and vomiting associated with mild abdominal distension are common side effects to this procedure. These may be counteracted by transient delay of the lavage process, or the administration of Metoclopropamide. Occasionally an orogastric tube is required. Care may be needed for the maintenance of adequate environment temperature control, since the fluid is administered chilled for palatability. This will prevent the hypothetical risk of inducing hypothermia to the patient due to the large intake of chilled fluid.

After the passage of fluid stools, it has been shown that the concentration of immunoglobulins in the WGLF remains stable over time (Ferguson et al, 1995). Thus having achieved a steady state and for convenience, the first clear specimen obtained is processed immediately as described in the following section (see 3.3.A.4. WGLF Processing)

This protocol was approved by the Medicine Subcommittee of the Lothian's Area Ethics Research Committee.

### **3.B.3.4. Whole Gut Lavage Fluid Processing**

#### **3.B.3.4a Preparation & Timing**

The need for immediate processing of the WGLF cannot be over-emphasised, since it has been shown that immunoglobulin and other proteins in the lavage fluid degrade rapidly. Within two hours there is a variable loss (0-62%) of immunoglobulin IgA activity as measured by an Enzyme linked immunosorbent assay (ELISA) technique (Gaspari et al, 1988). Furthermore, inhibitors used in the processing have been shown not to interfere with the ELISA assays which are subsequently performed on WGLF (Croft, 1996a).

In order to ascertain that the processing was done on time, a continuous monitoring of the timing of lavage handling took place. This also meant that, whenever a whole gut lavage was performed in other departments apart from the Gastrointestinal Laboratory at the Western General Hospital, the provision of facilities for processing on site was mandatory.

#### **3.B.3.4b. Processing Of WGLF & Handling**

20 ml of the WGLF were obtained from each patient. 10 ml of those were filtered through a glass filter (GAF/A, Whatman, Maidstone, UK). The following reagents were then added to 5 ml of filtered and 5 ml of unfiltered WGLF:

- i. 0.5ml of Soya bean trypsin inhibitor in PBS (SBTI).
- ii. 0.28ml of sodium ethylene diamine tetracetic acid in PBS (EDTA)
- iii. 0.12 ml phenyl methyl sulphonyl fluoride in 95% ethanol (PMSF)
- iv. 0.06 ml of sodium azide as a preservative
- v. 0.3ml of new-born calf serum (2 minutes later).

Extra samples were stored of the filtered and the unfiltered WGLF without the processing reagents, but with the addition of 0.03 ml of sodium azide in 1.5 ml of fluid. All specimens, processed or not, were stored in aliquots at  $-70^{\circ}\text{C}$ . If immediate storing was not possible, the specimen was transported stored in dry ice. After processing, the PEG content of the lavage fluid was reduced from



59g/L to 47.5g/L. As a result, the fluid stored would be 78.5% PEG-electrolyte solution and 21.5% gut secretions (Gaspari et al, 1988).

### **3.B.3.5. WGLF Assays**

The assays that are performed on the WGLF are described in detail in appendix I (see section 3.C). In brief, WGLF IgG is assayed by ELISA using affinity-purified goat anti-human IgG (North East Laboratories). The WGLF albumin and  $\alpha$ 1-antitrypsin are analysed by immunoturbidimetry; anti-human albumin was supplied by the Scottish Antibody Production Unit and anti-human  $\alpha$ 1-antitrypsin was obtained from the Protein Reference Unit.

Haemoglobin was assayed by the HemoQuant method using as a standard a simulated lavage fluid with PEG solution and human haemoglobin.

The elastase activity was assayed by its amidolytic effect on the substrate pyro Glu-Pro-Val-pNA. The cytokines IL-1 $\beta$  and IL-8 were assayed by ELISA kits from Cistron Biotechnology and R&D Systems.

## **3.B.4. Whole Gut Lavage In Ileoanal Pouches**

### **3.B.4.1. Introduction - The Protocol**

The general principles of the Whole Gut Lavage were applied to the study of the patients with an ileoanal pouch. The patients were first seen in the outpatient department, except from the inpatients, where the procedure was explained to them. Those who agreed were booked for the investigation on another day.

Patients were asked to fast overnight, but were instructed to take their medications as usual. When they arrived at the investigation suite in the morning, the procedure was again explained to them. The following steps were then followed :

- a. Whole Gut lavage
- b. History taking
- c. Blood taking

#### d. Pouchoscopy & biopsy

The results of the tests were forwarded to the referring Surgeon.

### **3.B.4.2. The Place & Time Of The Study**

The study took place in the investigation room, GI annexe of the Gastrointestinal Laboratory at the Western General Hospital or ward 39 and the attached endoscopy suite at the Centre of Liver and Digestive Disorders (CLDD) at The Royal Infirmary of Edinburgh. The inpatients were studied in the corresponding Surgical wards of the two Hospitals.

All of the outpatient whole gut lavages were performed in a morning session; 65% of them whilst an outpatient list of colonoscopies was taking place. Within the time limits of this morning session the pouch patients would have received their bowel preparation which was followed by pouchoscopy.

The five inpatients had their whole gut lavage during the hours of the early afternoon, having had an early breakfast.

### **3.B.4.3. Whole Gut Lavage Adverse Effects**

None of the whole gut lavages performed on the 71 patients had to be abandoned because of ill effects to the patients. There were no serious adverse events noted during the test, apart from transient nausea and 2 cases of vomiting. Four patients in total required an orogastric tube: 2 patients with vomiting and 2 patients who were intolerant to the taste of the Klean- Prep®.

### **3.B.4.4. Whole Gut Lavage Rate In Pouch Patients**

The whole gut lavage rate is defined as the amount of consumption of PEG solution per hour, till the patient produces a clear specimen. This clear specimen has been validated as a gut perfusate (Ferguson et al, 1995); the lavage rate on that study was 1 litre per hour.

Data on 67 pouch patients showed that the median consumption of Klean Prep during the lavage was 1.45 litre (IQ range: 1.25-1.8). The time required for completion of the lavage was 75 minutes (IQ range: 55-90). In the 67 pouch

patients studied the rate was 1.2 litre/hour (IQ range: 0.96-1.5). The initial diagnostic category did not affect the rate of the lavage.

There seemed to be no relation of the lavage rate to the current use of antidiarrhoeal medication, or to the dose of antibiotics, e.g. metronidazole.

### **3.B.5. Patient Questionnaire**

A structured clinical questionnaire was used to assess the patients' clinical status at the time of their gut lavage. This questionnaire (see appendix III, section 3.C) was subdivided in the following 3 separate entities:

#### **3.B.5.1. Patient Component Of The Questionnaire**

Information about the following were recorded:

- a. patients demographic details(age, sex)
- b. preoperative diagnosis and time when the diagnosis was first established
- c. past medical and surgical history
- d. family history
- e. medication prior to the colectomy
- f. extraintestinal manifestations

#### **3.B.5.2. Operation Component Of The Questionnaire**

The following parameters were recorded:

- a. date of the colectomy, date of the construction of the pouch and the time of closure of the covering ileostomy, if performed
- b. type of pouch: J,W, or S (information obtained from case notes)
- c. type of anastomosis, performance of mucosectomy or not
- d. postoperative complications (dehiscence, pelvic sepsis, peritonitis, anastomotic stricture, perineal sepsis, fistula, outlet obstruction, intestinal obstruction sexual or micturition disturbances)
- e. the immediate post-operative medical treatment
- f. post-operative extra-intestinal manifestations, and lastly

- g. histology report of proctocolectomy reviewed, to check for:
- i. stigmata of indeterminate colitis
  - ii. extend of the disease
  - iii. evidence of dysplasia or atypia and its degree
  - iv. state of the appendix in the resected specimen

### **3.B.5.3. 'Functional' Component Of The Questionnaire**

A modification of Öresland Functional criteria was used to assess the present function of the patients' pouch and their 'best ever' function (Öresland et al 1989; see Table 2.1 chapter II). The following were recorded:

- a. bowel function during the day and night
- b. faecal urgency (inability to defer defaecation for 30 minutes)
- c. evacuation difficulties (straining or spending >15 minutes at the toilet at any point during the week)
- d. faecal incontinence or spotting
- e. usage of protective pad (day- or night-time)
- f. dietary restrictions
- g. medications
- h. presence of perianal soreness
- j. any social handicap

At the end, a functional score was compiled both for the present time and the time of the pouch's 'best ever' function. A score was recorded from 0-15, where 0=best and 15=worst 'functional' result.

### **3.B.6. Haematology & Clinical Chemistry Results**

The following parameters were recorded:

- a. Full Blood Count
- b. Erythrocyte Sedimentation rate (ESR)
- c. C-reactive protein (CRP)
- d. Serum Immunoglobulin plasma levels

The specimens were assayed either in the Western General Hospital or the Royal Infirmary of Edinburgh Haematology, Clinical Chemistry or Microbiology laboratories and it was included in the routine follow up of these patients.

### **3.B.7. Pouchoscopy & Histology**

Pouchoscopy rigid or flexible was performed. Pouchoscopies were performed in the GI Annexe for the outpatients, or at the endoscopy suite at the CLDD, or in ward 39 at the Royal Infirmary. Patients were positioned in the left lateral position. Written or verbal consent was obtained in all cases. The presence of oedema, granularity, friability, loss of vascular pattern, mucous exudate and ulceration was noted. Multiple biopsies were taken from the most inflamed area or, if no overt inflammation was obvious, from a representative part of the pouch, normally posteriorly at 10 cm from the anal verge. This area was chosen since histological findings showed this area to have the maximal changes (Shepherd et al, 1993). Patterson biopsy forceps were used, when performing rigid pouchoscopy, and ordinary flexible sigmoidoscopy biopsy forceps were used during flexible pouchoscopy. Rigid scopes were used in 37% of the cases. The pouchoscopy score was not different between flexible or rigid pouchoscopy ( $p>0.05$ , Mann-Whitney test). It was of particular interest to notice that the bowel preparation and subsequently the views obtained were immaculate.

The histological specimens obtained were fixed in formalin, and then sent to the histopathology service of both the Western General Hospital and the Royal Infirmary. Information about the patient was given to the pathologists.

### **3.B.8. Pouchitis Disease Activity Indices**

#### **3.B.8.1. Introduction**

Various scoring systems and criteria have been proposed over the years to assess the 'disease activity' in pouches. In this study two of them were used: the Pouchitis Disease Activity Index (PDAI); and the Moskowitz criteria. The rationale behind this approach was that each one of these indexes had its own advantages

and problems, but studies comparing both of them are really very few. This is particularly true for the newer one, the Pouchitis Disease Activity Index (PDAI), which may still require more research.

### **3.B.8.2. Calculation Of The PDAI**

This is a composite index devised by the Mayo Clinic group (Sandborn et al 1994). The advantage of this index is that it forms a continuum from 0-18, while a score  $\geq 7$  signifies pouchitis. PDAI has already been described in chapter II (see Table 2.4).

The clinical features of the PDAI include stool frequency, bleeding, urgency or abdominal cramps, and fever above  $37.8^{\circ}\text{C}$ . Noticeably it is not the absolute frequency that matters, but the difference between the current frequency and the usual post-op frequency. The latter is rather subjective and may impose difficulties because of that. The clinician has to rely on patient's ability to note this difference according to their psychology at the time.

The endoscopic criteria derive 1 point each by their presence. These include oedema, granularity, friability, loss of vascular pattern, mucous exudate, and ulceration.

The histological criteria make no effort to record any of the chronic inflammatory changes or the villous atrophy that sometimes is seen in pouches. Instead they only rely on the polymorphonuclear infiltration and the ulceration. This is because, it has been shown that symptomatology correlates with acute inflammatory features but not with chronic inflammation (Moskowitz et al, 1986). The score varies from 0-3 and corresponds to the scoring of Moskowitz. By definition, for scoring purposes the most inflamed area is recorded.

At the time of the gut lavage the clinical and endoscopic score would be filled in. The histological score is filled in later based on the actual histological report. The PDAI was calculated in advance to avoid any bias from knowing the gut lavage parameters.

### **3.B.8.3. The Moskowitz Criteria**

The Moskowitz criteria define pouchitis based on clinical, endoscopic and histological criteria. Pouchitis is characterised by diarrhoea exceeding 6 times a day, endoscopic score greater than 4 on a scale similar to the PDAI and histopathological features of neutrophilia and ulceration exceeding 4 on a histological scale. This definition also adopts an 'all or none' approach, since the patient has to fulfil all three criteria before pouchitis is held to be present. It is very similar to the PDAI, but follows a binary concept and not that of a continuum. For many investigators, it has become the '*gold standard*' in diagnosis of pouchitis (Moskowitz et al 1986).

### **3.B.9. Statistical Analysis & PC Software**

The data on this thesis were stored using the Access version 2.0 (Microsoft), whilst statistical analysis was performed using the Minitab for Windows version 10 (Minitab statistical software) and the Excel version 5.0 (Microsoft). Graphs were produced using Prism version 1.03 (Graphpad). The word processing was performed with Word for Windows version 6.0 (Microsoft), whilst the references were prepared with the Reference Manager for Windows version 6.01 (Research Information Systems).

Some data has been presented as percentages. For descriptive statistics, the median with the interquartile range and the range is given. Comparisons between parameters of different groups have used the non-parametric Mann-Whitney test. The correlation between various parameters used the Pearson correlation coefficient and statistical significance was achieved at  $p < 0.05$ . At each chapter, the specific statistical tests used are individually referred to as applied.

### **3.B.10. The IBD & Ileostomy Database**

The Gastrointestinal Laboratory maintains a database on patients who had a Whole Gut Lavage. Part of this database is the IBD database which includes anatomical characteristics of the patients, their haematological parameters of disease activity (haemoglobin, white cell count, platelet count, C- Reactive Protein

and ESR), their lavage parameters and clinical appraisal of their disease. This database includes also patients with ileostomies. These data are collected in a prospective manner and stored in a Dbase III program on a PC.





# **CHAPTER IV**

## **PATIENTS' CLINICAL FEATURES**

## **4.1. INTRODUCTION**

In this chapter the clinical, endoscopic and histological characteristics of the patients, studied by Whole Gut Lavage, are described. The majority of the patients had previous restorative proctocolectomy for ulcerative colitis (UC). A few patients underwent restorative proctocolectomy for causes other than inflammatory bowel disease (see chapter III). The study of the clinical parameters on these patients would allow a more accurate description of patients' health state at the time of the gut lavage. This will also allow the study of possible connections between the various clinical and laboratory parameters.

## **4.2. PATIENTS & METHODS**

### **4.2.1a. Patients**

Seventy-four consecutive patients attending the General Surgical Departments of the Royal Infirmary and the Western General Hospital of Edinburgh were recruited for study. Five patients were excluded as described in chapter III (section 3.2.3.1). Three more patients had to be excluded from the analysis, because the procedure followed did not comply fully with the protocol. Consequently 71 patients were analyzed.

There were 40 male patients and 31 female. Age ranged from 18.2 to 76.4 years (median: 41.7 years). The period of time between the study and the creation of pouch and restoration of intestinal continuity ranged from 1.1 to 83.7 months post operatively (median: 26.9 months).

### **4.2.1b. Patient Subgroups**

Patients were subdivided according to the original diagnosis for which the pouch was created. From the 71 patients, 61 patients had UC, 5 had indeterminate colitis (IC), and 5 patients other diseases - refractory constipation, familial adenomatous polyposis coli (FAP) or multiple synchronous cancers (see chapter III: 'Non-IBD group', sections 3.A.4.1, 3.A.4.2, 3.A.4.3).

In the early stages of the study another subdivision became apparent. Several patients were identified who had recent pouch construction for UC. A cut off point of six months was taken to separate this 'early' group, which corresponded to the differences in the clinical profile observed for these patients. This group numbered five patients.

The characteristics of these groups are described in Table 4.1. The 'non IBD' group included 2 patients who had their colectomy for multiple synchronous cancers, one patient with FAP, and two who had their colectomy for intractable constipation, after failure of ileorectal anastomosis. The term 'median time post pouch' is defined as the time between my study and patient's intestinal continuity restoration after pouch surgery.

### **4.2.2a. Methods**

Detailed analysis of the protocol followed is given in chapter III. In brief, the patients attended for whole gut lavage study. During this time medical history was obtained via a prestructured questionnaire (see chapter III, section 3.B.5). Blood tests were performed for full blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum immunoglobulins (IgG, IgM, IgA). This was followed by pouchoscopy and biopsy as described earlier (see chapter III, sections 3.B.6, 3.B.7, 3.B.8).

### **4.2.2b. Statistical Analysis**

The statistical analysis of the results used the median values; 95% confidence interval (CI) or range; the Pearson correlation coefficient and its p value; the non-parametric Mann-Whitney U test for unpaired comparison of

**Table 4.1.** Demographic details of the 71 patients with ileoanal pouch who participated in the study. The pouch groups included: the 'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis; the 'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months; the 'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis; and the 'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD. There were no significant differences in gender or age between the four groups. No significant difference in the median time post pouch was seen between the three groups - 'UC', 'non IBD', and IC.

<b>Pouch groups</b>	<b>Male</b>	<b>Female</b>	<b>Age</b>	<b>median time post pouch</b>
UC group (n = 56)	32	24	42.8 (18.2-74.6)*	28.9**
'early' group (n=5)	4	1	36.3 (23.0-35.0)	3.4
IC group (n = 5)	1	4	43.7 (35.7-61.0)	26.5
non IBD group (n = 5)	3	2	48.2 (23.2-76.8)	25.6

The results are expressed as: median values (range).

\*Age is expressed in years.

\*\*Median time post pouch is expressed in months.

observations;  $\chi^2$  with Yates correction with Odds Ratio (OR) or Relative Risk (RR). P values less than 0.05 were considered significant.

## **4.3. RESULTS**

### **4.3.A. Patients Characteristics**

#### **4.3.A.1. Demographic Characteristics**

Demographic characteristics are given in detail in Table 4.1.

#### **4.3.A.2. Family History**

Ten patients (16%) in the UC group had a family history of IBD. Eight of these (13%) had first degree relatives with IBD. Nineteen patients had family history of other diseases (see Table 4.2). Nine patients had a family history of bowel, lung, cervical, liver and breast cancer. There was only one patient in IC group with family history of IBD, but four patients had significant family history (see Table 4.2). There was no family history of IBD in the 'non IBD' group. There was no difference in the family history of IBD between UC and IC cases. UC and IC patients had similar number of first degree relatives with IBD. There was no difference in ischaemic heart disease (IHD) or cancer family history between IBD and 'non IBD' patients.

#### **4.3.A.3. Age At Presentation & Age At Colectomy**

The age of presentation in the various groups is presented in Figure 4.1. Seven patients (10.4%) with IBD were older than 50 years at presentation. In the 'non IBD' group the presentation age was variable. This may be explained by

**Table 4.2.** The family history of the various pouch groups. The family history includes first degree relatives with IBD and details of other family history.

Pouch groups	FHx of IBD	IBD in 1st degree relatives	Other FHx
UC group (n = 56)	9	7	9 cancer 4 asthma 2 diabetes 3 IHD (17 patients)*
'early' group (n = 5)	1	1	1 IHD (1 patient)*
IC group (n = 5)	1	1	1 cancer 1 asthma 1 diabetes 2 IHD (4 patients)*
non IBD group (n = 5)	0	0	1 FAP 1 Spina Bifida 1 IHD (3 patients)*

'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis;

'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months;

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis;

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD

\* overall number of patients with other family history.

FHx: family history.

the heterogeneity of the 'non IBD' group: 2 patients had severe constipation, one had FAP, and the two older patients had multiple synchronous carcinomas.

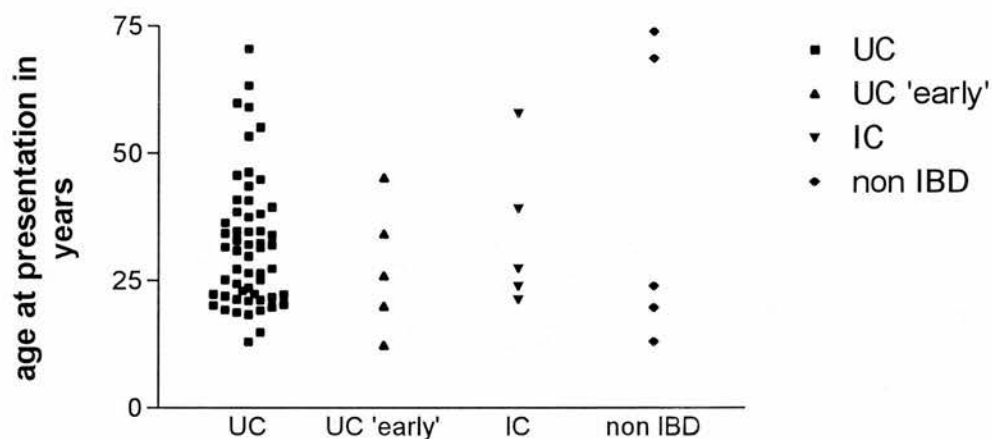
The time between presentation and colectomy is presented in Figure 4.2. This ranged from 1.0 month to 29.6 years (median: 6.9 years) in the UC patients. In the IC patients, this ranged from 1.0 month to 19.5 years (median: 8.6 years).

#### **4.3.A.4. Past Medical & Surgical History**

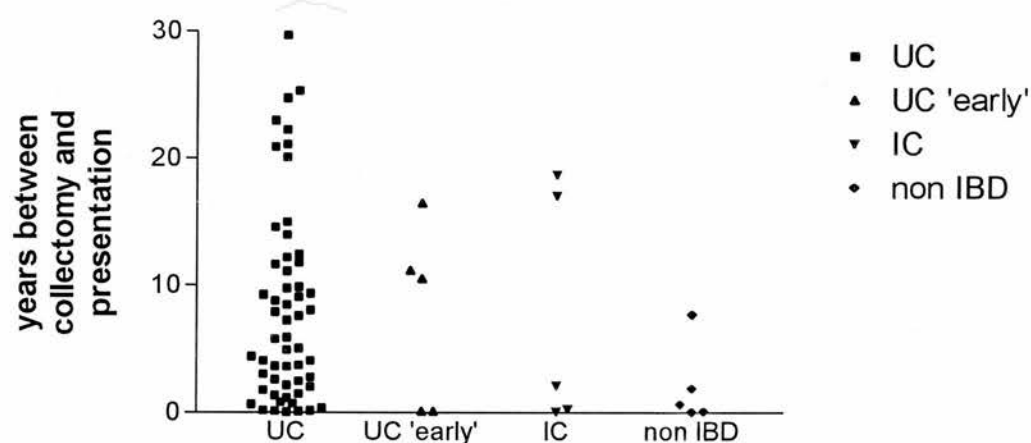
Six patients had previous tonsillectomy in the UC group (10%) and one in the 'non IBD' group. The incidence of appendectomy in the UC group was 5%. Anal and pelvic surgery (hysterectomy or sterilization) was present in 5% and 3% respectively of patients. Three patients had a history of urolithiasis (5%). There were a variety of chest complaints, hypertension, IHD and psychotic problems related to steroids in one case (see Table 4.3).

#### **4.3.A.5. Medications Pre-Colectomy**

The drug regimens followed immediately pre-colectomy in the UC group are shown in Table 4.4. Preoperative treatment was unknown in three patients. One patient was on no treatment except iron, due to drug intolerance. 17% of patients were on high dose of systemic steroids (more than 30mg Prednisolone or equivalent). All of these patients had pancolitis and all but two had emergency operations. 41% of patients were on steroids and 5-aminosalicylates (ASA). This group included a fair number of left colitis (35%) and only 54% required emergency operations. 24% of patients were maintained only on 5-ASA. Although 79% of these patients underwent elective surgery, 43% exhibited a degree of dysplasia, including one case of Dukes C cancer. Finally, 14% of patients were maintained on azathioprine. This included one patient who has been on azathioprine alone, the rest being on combination of azathioprine and steroids. 75% of patients underwent elective operations. 25% of this group had left sided colitis. Dysplasia was exhibited in 25%.



**Figure 4.1.** Patients' age at presentation of diagnosis of the various pouch groups: 'the 'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis; the 'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months; the 'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis; and the 'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD.



**Figure 4.2.** The period between presentation and colectomy in the various pouch groups expressed in years.



**Table 4.3.** The past medical history in the various pouch groups. The surgical, medical and psychiatric history are presented separately.

Pouch groups	Surgical PMHx	Medical PMHx	Psychiatric PMHx
UC group (n=61)	tonsillectomy (6)* appendicectomy (3) anal surgery (3) hysterectomy (2) breast surgery (4) fractures 8 kidney stones (3)	Tuberculosis (2)* asthma ((3)) pneumonia (2) pleurisy (2) bronchiectasis (2) diabetes (2) pancreatitis (2) hypertension (2)	alcohol abuse (2)* psychotic history (3)
IC group (n=5)	hysterectomy (1)	diversion colitis (1)	
non IBD group (n=5)	tonsillectomy (1) fractures (2)	meningitis (1) epilepsy (1)	neurosis (2)

‘UC group’ - patients who underwent restorative proctocolectomy for ulcerative colitis;

‘early group’ - patients with restorative proctocolectomy for UC within the last 6 months: included in UC group

‘IC group’ - patients who underwent restorative proctocolectomy for indeterminate colitis;

‘non IBD group’ - patients underwent restorative proctocolectomy for causes other than IBD

The number of patients suffering from the given disease is presented in brackets(\*)

PMHx: past medical history

**Table 4.4.** Precolectomy treatment in the pouch groups. Patients were treated with local or systemic steroid medication with or without 5-aminosalicylates. A number of patients were treated with azathioprine or cyclosporin prior to restorative proctocolectomy.

<b>Pouch groups</b>	<b>Steroids</b>	<b>5ASA &amp; Steroids</b>	<b>5ASA</b>	<b>Azathioprine combinations</b>	<b>Cyclosporin</b>	<b>Local Steroids</b>
UC (n = 58)	10	24	14	8	1	17
IC (n = 5)	2	2	1			

'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis;

'early group' - patients with restorative proctocolectomy for UC within the last 6 months: included in UC group

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis;

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD

No information available on 3 UC patients.

5ASA: 5-Aminosalicylates.

### **4.3.A.6. Extraintestinal Manifestations**

The incidence of extraintestinal manifestations in the preoperative period was 21.3%, whereas in the postoperative period was 11.4% (de novo 3.2%). Sclerosing cholangitis was noted in 5% of the patients; eye problems such as iridocyclitis or uveitis in 3% (2 patients). Joint pathology appeared more common in 21%. There was one case of pyoderma gangrenosum and two of erythema nodosum (5%). The patient with pyoderma gangrenosum and one of the patients with the erythema nodosum continued to have the problem postoperatively. In the IC group only one patient had previous history of polyarthralgia. See Table 4.5.

### **4.3.A.7. The Histology Of Resected Colon Specimens**

The histological aspects in the 'non IBD' group confirmed the initial diagnosis for which the colectomy was performed. There were two patients with synchronous cancers, two patients were treated surgically for intractable constipation and one patient with FAP. The histopathology in the UC and IC groups is described below.

#### **4.3.A.7a Histological Extent Of Disease**

The extent of the disease was based on the histology report of the resected specimen. In the UC group 22% of patients had left sided colitis. All IC patients had pancolitis. See Table 4.6.

#### **4.3.A.7b Backwash Ileitis**

The incidence of backwash ileitis is shown in Table 4.7. The incidence in the UC group was 17%. In the IC group only 1 patient was noted to have backwash ileitis.

**Table 4.5.** The extraintestinal manifestations in the various pouch groups. There was no difference between UC & IC groups at the total number of EIM exhibited.

Pouch groups	EIM	Description of Manifestations
UC group (n=56)	17	3 Sclerosing cholangitis 1 uveitis 1 iridocyclitis 1 pyoderma gangrenosum 10 joint involvement 1 liver function abnormalities
'early' group (n=5)	3	2 erythema nodosum 3 joint pains
IC group (n=5)	1	1 arthritis

'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis;

'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months;

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis;

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD

The number of patients suffering from every and each manifestation are given in the description of manifestations.

EIM: extraintestinal manifestations.

**Table 4.6.** The extent of disease is presented in the pouch groups\*. There was no significant difference in the extent of disease between the two groups.

<b>Pouch groups</b>	<b>Pancolitis</b>	<b>Left sided colitis</b>
UC group (n=60)	47	13
IC group (n=5)	5	0

**Table 4.7.** The presence of backwash ileitis in the UC and IC pouch groups\*. There was no significant difference between the two groups.

<b>Pouch groups</b>	<b>Backwash Ileitis</b>	<b>Unknown</b>
UC group (n=60)	8	15
IC group (n=5)	1	1

\*‘UC group’ - patients who underwent restorative proctocolectomy for ulcerative colitis;

‘early group’ - patients who underwent restorative proctocolectomy for UC within the last 6 months;

‘IC group’ - patients who underwent restorative proctocolectomy for indeterminate colitis;

Record of extent of disease or presence of backwash ileitis was missing in one patient’s histological report, though other histological features were described.

**Table 4.8.** The state of the appendix in the various pouch groups\*.

<b>Pouch groups</b>	<b>Normal</b>	<b>Inflamed</b>	<b>Fibrotic</b>	<b>Unknown</b>
UC group (n=60)	5	14	14	28
IC group (n=5)	0	1	3	1
non IBD group (n=5)	2	0	0	3

**Table 4.9.** The presence of dysplasia, atypia and cancer in resected specimen in various pouch groups\*. There was no significant difference between the groups.

<b>Pouch groups</b>	<b>Dysplasia</b>	<b>Atypia</b>	<b>Ca colon</b>	<b>Normal</b>	<b>Unknown</b>
UC group (n=60)	12	6	1	36	5
IC group (n=5)	0	1	0	3	1
non IBD group (n=5)	1	0	2	2	0

\*'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis

'early group' - patients with restorative proctocolectomy for UC within the last 6 months: included in UC group

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD

Record of the state of appendix & presence of atypia was missing in one patient's histology report

#### **4.3.A.7c The Pathology Of The Appendix**

The appendix in the resected specimen was frequently abnormal, showing either inflammation, or fibrosis. This was also true for patients who had left sided colitis. The state of the appendix was not described in some patients. The occurrence of the various abnormalities relating to the pathology of the appendix is given in Table 4.8.

#### **4.3.A.7d Dysplasia In The Resected Specimens**

Eighteen per cent of the resected specimens showed evidence of dysplasia. The incidence for observed cancer was 1.5%.

#### **4.3.A.8. The Operative Characteristics**

The operations were performed at the Royal Infirmary of Edinburgh and the Western General Hospital in Edinburgh. There were few patients who had their initial colectomy elsewhere in Scotland but referred for restoration or follow up in Edinburgh. The operations were performed by Messrs. Bartolo (57 patients), Hamer-Hodges (11 patients), Smith (1 patient) and Keenan (1 patient). All the patients but one had their operation in NHS hospitals.

#### **4.3.A.8a Number Of Emergency Or Urgent Operations**

Forty-five per cent of the operations in the UC group were performed as urgent (see Table 4.10). There was no higher complication rate of urgent operations compared with elective procedures. As expected, fewer restorative proctocolectomies were performed as one stage operations, when the colectomy was urgent ( $\chi^2 = 8.6$ ,  $p = 0.003$ ).

**Table 4.10.** The number of urgent colectomies performed in each pouch group\*. There was no significant difference between the UC & IC groups.

<b>Pouch group</b>	<b>Number of Urgent Colectomies</b>
UC group (n=61)	28
IC group (n=5)	4
non IBD group (n=5)	2

**Table 4.11.** The number of operations before restoration of continuity in the various pouch groups\*. There was no significant difference between the groups.

<b>Pouch groups</b>	<b>One stage</b>	<b>Two stages</b>	<b>Three stages</b>
UC group (n=61)	30	26 (3 with IRA)	5
IC group (n=5)	1	4 (no IRA)	0
non IBD group (n=5)	2	3 (2 with IRA)	0

\*'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis

'early group' - patients with restorative proctocolectomy for UC within the last 6 months: included in UC group

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD

IRA : ileorectal anastomosis



**Table 4.12.** The distribution of ‘J’, ‘W’ and ‘S’ pouch in the various pouch groups\*. The characteristics in the presenting disease were similar in both subgroups with ‘J’ or ‘W’ pouches, in the UC group.

Pouch groups	‘J’ pouch	‘W’ pouch	‘S’ pouch
UC group (n=61)	48	12	1
IC group (n=5)	5	0	0
non IBD group ( n=5)	4	1	0

**Table 4.13.** Number of complications in the various pouch groups. There was no significant difference between the groups.

Pouch groups	C <sub>0</sub>	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>total</sub>	C/patient
UC group (n=61)	29	21	9	2	32 (52%)	0.7
IC group (n=5)	1	2	2	0	4 (80%)	1.2
non IBD group (n=5)	2	2	1	0	3 (60%)	0.8

‘UC group’ - patients who underwent restorative proctocolectomy for ulcerative colitis

‘early group’ - patients with for UC within the last 6 months: included in UC group

‘IC group’ - patients who underwent restorative proctocolectomy for indeterminate colitis

‘non IBD group’ - patients underwent restorative proctocolectomy for causes other than IBD

Complications: C; C<sub>0</sub> =no complications, C<sub>1</sub> =1 complication, C<sub>2</sub> =2 complications, C<sub>3</sub>=3 complications,

C<sub>total</sub>=total number of complications (percentage); C/patient: complication per patient

**Table 4.14.** The various complications in the pouch groups\*.

<b>Pouch groups</b>	<b>UC group (n=61)</b>	<b>IC group (n=5)</b>	<b>non IBD group (n=5)</b>
<b>Complications (total)</b>	47	6	4
<b>Dehiscence anastomosis</b>	5	0	0
<b>Pelvic sepsis</b>	1	0	0
<b>Anastomotic stricture</b>	9	1	0
<b>Peritonitis</b>	4	0	1
<b>Perineal sepsis</b>	1	0	0
<b>Fistula</b>	2	1	0
<b>Outlet obstruction</b>	2	0	0
<b>Intestinal obstruction</b>	9	1	0
<b>Other</b>	16	3	3

\*‘UC group’ - patients who underwent restorative proctocolectomy for ulcerative colitis

‘early group’ - patients with restorative proctocolectomy for UC within the last 6 months: included in UC group

‘IC group’ - patients who underwent restorative proctocolectomy for indeterminate colitis

‘non IBD group’ - patients underwent restorative proctocolectomy for causes other than IBD

#### **4.3.A.8b Operative Stages**

Forty-nine per cent of the patients had their operation as one stage procedure, in the UC group. As mentioned above, patients who underwent emergency colectomies tended to have staged operations in order to create and then close a covering ileostomy. Since the creation of an ileostomy may present with complications of its own, patients who had staged operations had higher postoperative complication rate ( $\chi^2=8.292$ ,  $p=0.004$ ). See Table 4.11.

#### **4.3.A.8c Pouch Design**

The type of pouch constructed is given in Table 4.12. As seen most of the pouches were of 'J' configuration, the 'W' being only 18% of the total. There was only one 'S' pouch. All but one of the 'J' pouches was constructed by the same surgeon, and all but one of the 'W' pouches was performed by another surgeon. The suture material differed between 'J' and 'W' pouches, the 'J' pouches were all stapled, and the 'W' were hand sewn with non absorbable suture material (Neurolon™).

The pouch anal anastomosis in most of the cases in both 'J' and 'W' groups were made by the use of the staple gun, slightly smaller diameter usually for the 'W' pouches. In spite of this, the incidence of anastomotic stricture in the UC group did not reach significance ( $p=0.051$ ). Only two anastomoses were hand sutured among the 71 patients. Equally only three mucosectomies of the anal cuff were performed.

#### **4.3.A.8d Postoperative Complications**

The following Tables 4.13 and 4.14 describe the number and type of complications noted in the various groups. For the 71 patients there were 5 cases of anastomotic dehiscence, 1 case of pelvic sepsis, 10 cases of anastomotic stricture, 5 cases of peritonitis, 1 case of perineal sepsis, 3 cases of fistulas, 2 cases of outlet obstruction and 10 cases of intestinal obstruction.

Other complications included:

- a) UC group: 2 pulmonary embolism, 3 wound infections, 1 incisional hernia, 2 chest infections, 1 acute psychosis, 3 persistent postoperative anaemia.
- b) IC group: 1 dyspareunia, 1 suprapubic abscess (fistula related).
- c) 'Non IBD' group: one bile leak from synchronous cholecystectomy.

Surgery was needed in 21 of the 71 total patients (re-operation rate: 29% of the patients; 37% of their complications). In the UC group as mentioned above, patients with staged operations were inclined to have more complications.

### **4.3.A.9. 'Functional' Characteristics**

The 'functional' characteristics for each patient were recorded using a questionnaire (see chapter III, appendix III). These were collated to produce a functional score (Öresland et al, 1989).

#### **4.3.A.9a Bowel Activity**

Figures 4.3 and 4.4 show the bowel activity during the day and during the night for the various groups. For the UC group, the bowel activity during the day ranged from 2 to 15 (median: 6.0; 95%CI: 5.5, 6.5). The bowel activity during the night ranged from 0 to 7 (median: 1.0; 95%CI: 1.0, 1.5).

#### **4.3.A.9b Pouch Related Difficulties:**

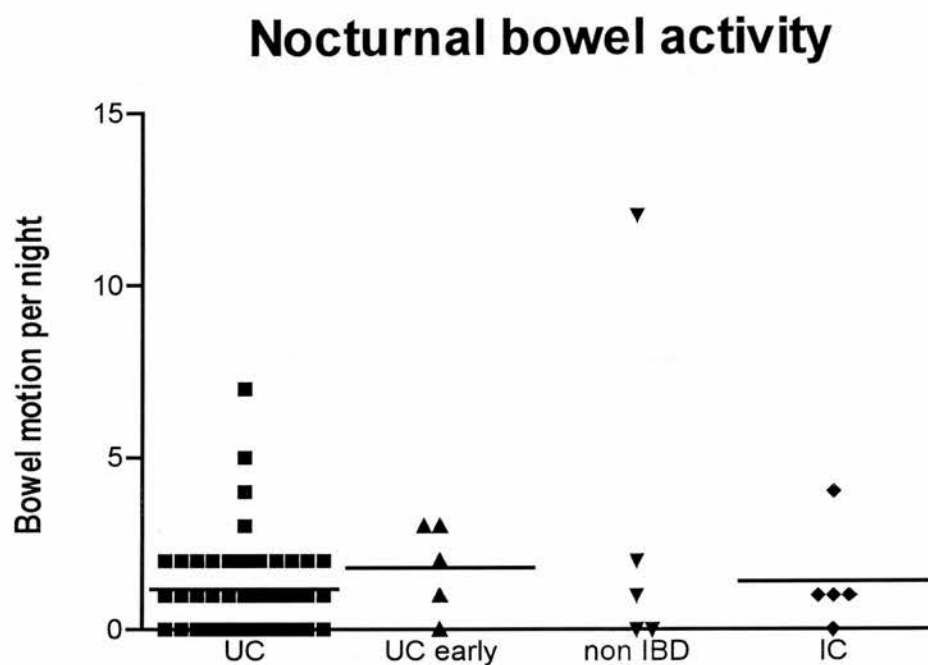
##### **Urgency - Difficult Evacuation - Perianal Discomfort - Social Handicap**

Table 4.15 summarizes the above mentioned aspects of function in all three groups. Since the UC group is the largest, it serves as the principle reference.

##### **UC Group**

- A. 15% (9 patients) exhibited urgency (15%). Patients were unable to withhold evacuation for more than 30 minutes.





**Figure 4.4.** Night time bowel activity in the various pouch groups: 'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis; the 'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months; the 'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis; and 'non IBD group' - patients underwent pouch surgery for causes other than IBD. The transverse lines in the figure represent the means of the values observed. There was no significant difference between the groups.

**Table 4.15.** Pouch related difficulties in the various pouch groups\*.

<b>Pouch groups</b>	UC group (n=61)	IC group (n=5)	non IBD group (n=5)
<b>Urgency</b>	9	2	3
<b>Evacuation difficulties</b>	13	2	2
<b>Perianal discomfort</b>	34	2	3
<b>Diet change</b>	23	2	2
<b>Social handicap</b>	16	0	3

The apparent higher urgency in the non IBD group, due to small numbers, did not reach statistical significance (with Yates correction). It resulted from the inclusion of two patients who had their operation for severe constipation.

**Table 4.16.** Incontinence and pad use in the various pouch groups\*. There was no significant difference between the three groups.

<b>Pouch groups</b>	<b>Day soiling</b>	<b>Night soiling</b>	<b>Pad/day</b>	<b>Pad/night</b>
UC group (n=61)	12	14	6	10
IC group (n=5)	1	1	1	0
non IBD group (n=5)	0	0	0	0

\*'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis

'early group' - patients with restorative proctocolectomy for UC within the last 6 months: included in UC group

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD

**Table 4.17.** The presence of bleeding per pouch as perceived by the patient in the various pouch groups: 'the 'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis; the 'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis; and the 'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD.

<b>Pouch groups</b>	<b>Bleeding per pouch</b>
UC group* (n=61)	17
IC group (n=5)	0
non IBD group (n=5)	2

**Table 4.18a.** Drug use in the various pouch groups.

<b>Pouch groups</b>	<b>Codeine</b>	<b>Loperamide</b>	<b>Metronidazole</b>	<b>Steroid</b>
UC group* (n=61)	16	11	15	6
IC group (n=5)	1	0	1	0
non IBD group (n=5)	1	0	0	0

\* - the 'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months included in the UC group.



**Table 4.18b.** Medications prescribed before the whole gut lavage study in the various pouch groups.

<b>Pouch groups</b>	<b>Antibiotics</b>	<b>Asacol</b>	<b>Methyl cellulose</b>	<b>aspirin or NSAIDs</b>	<b>Iron</b>	<b>other (number)</b>
UC (n=61)	3	3	2	3	1	Lomotil (1) Digoxin(1) antidepressants(1) capoten(1) creon(1) thyroxine(1) stemetil(1)
IC (n=5)					1	progesterone(1) trimovate cream(1)
non IBD (n=5)				2		carbamazepine(1) praxilene(1)

\*‘UC group’ - patients who underwent restorative proctocolectomy for ulcerative colitis

‘early group’ - patients with restorative proctocolectomy for UC within the last 6 months: included in UC group

‘IC group’ - patients who underwent restorative proctocolectomy for indeterminate colitis

‘non IBD group’ - patients underwent restorative proctocolectomy for causes other than IBD

- B. 21% were recorded as having evacuation difficulties, i.e. straining at anytime during the week, or a toilet 'session' lasting more than 15 minutes.
- C. 56% complained of perianal discomfort .
- D. 38% of patients admitted a change in diet after surgery.
- E. 26% considered that the pouch function interfered with their activities and life style.

#### **4.3.A.9c Pouch Continence & Use Of Incontinence Pads**

The patients were asked whether they experienced any episodes of faecal incontinence for solid or liquid stool, or had troublesome soiling, either during the day or at night (Table 4.16). In the UC group, the incidence of faecal incontinence was 20% during the day and 23% during the night. 8% of the UC patients were incontinent during both day and night. Only 10% and 16% of the patients would rather wear a protective pad for the day or for the night as a permanent precaution. Patients with daytime soiling require a day pad, but may not require a night pad ( $\chi^2=8.168$ ,  $p=0.0004$ ). On the contrary, patients who complain of night soiling, require protective pads for both day and night ( $\chi^2=8.168$ ,  $p=0.004$  and  $\chi^2=20.223$ ,  $p=0.0001$  respectively).

#### **4.3.A.9d Bleeding Per Pouch**

Seventeen (28%) UC patients reported some form of pouch bleeding. No IC patients and only 2 in the 'non IBD' group complained of pouch bleeding (see Table 4.17).

#### **4.3.A.9e Medications**

The most commonly used drugs were antidiarrhoeal such as codeine and loperamide, and metronidazole. Only one patient was on Lomotil. Some patients also used methylcellulose and steroids either local or systemic.

In the UC group, 26% were on codeine (Table 4.18a). In the same group, 18% were on loperamide. Only two patients were using both drugs. Metronidazole was used in 25% of patients. The rest of the medications are mentioned in the Tables 4.18a and 4.18b. Systemic steroid treatment was reported in two cases: for treatment of florid postoperative extraintestinal manifestation (pyoderma gangrenosum) in one case, and for active pouchitis in another case. One patient used local steroids for treatment of pouchitis. One patient in the 'early' group required systemic steroids for erythema nodosum. In the same group, two patients used local steroids.

#### **4.3.A.9f 'Functional' score**

The 'functional' score used was an adaptation of the Öresland scoring system (Öresland et al, 1989). Figure 4.5 shows the distribution of scoring between the groups.

### **4.3.A.10. Haematological Parameters**

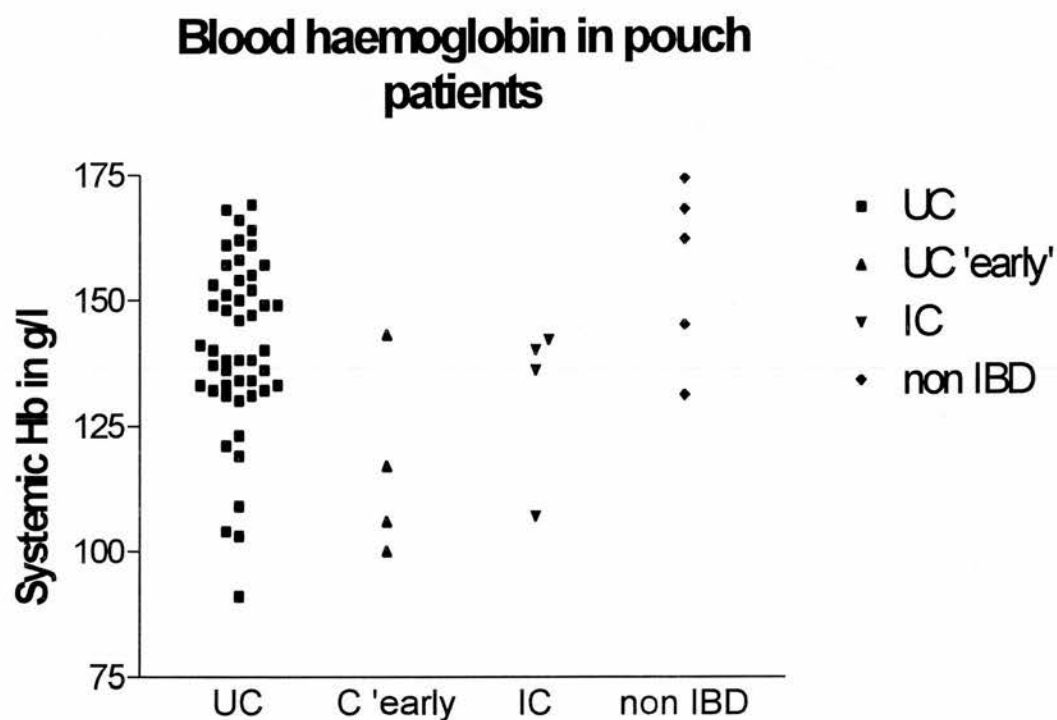
#### **4.3.A.10a Blood Haemoglobin**

The systemic haemoglobin values of the patients studied are presented in Figure 4.6. The haemoglobin in the UC group ranged from 91.0 to 169.0 g/L (median: 142.0; 95%CI: 136.0, 147.0). The haemoglobin in the 'early' group was lower (median: 111.5g/L;  $p=0.034$ , ). Female patients had lower haemoglobin compared to men in the UC group ( $p=0.00001$ ).

#### **4.3.A.10b Total White Cell Count (WCC)**

The systemic WCC in the UC group ranged from  $9.1 \times 10^9/L$  to  $15.5 \times 10^9/L$  (median:  $7.0 \times 10^9/L$ ; 95%CI 6.1, 7.9). This was not statistically different from the WCC in the 'early' group. See Figure 4.7.





**Figure 4.6.** Estimation of blood haemoglobin (Hb) in pouch patients' groups\*.

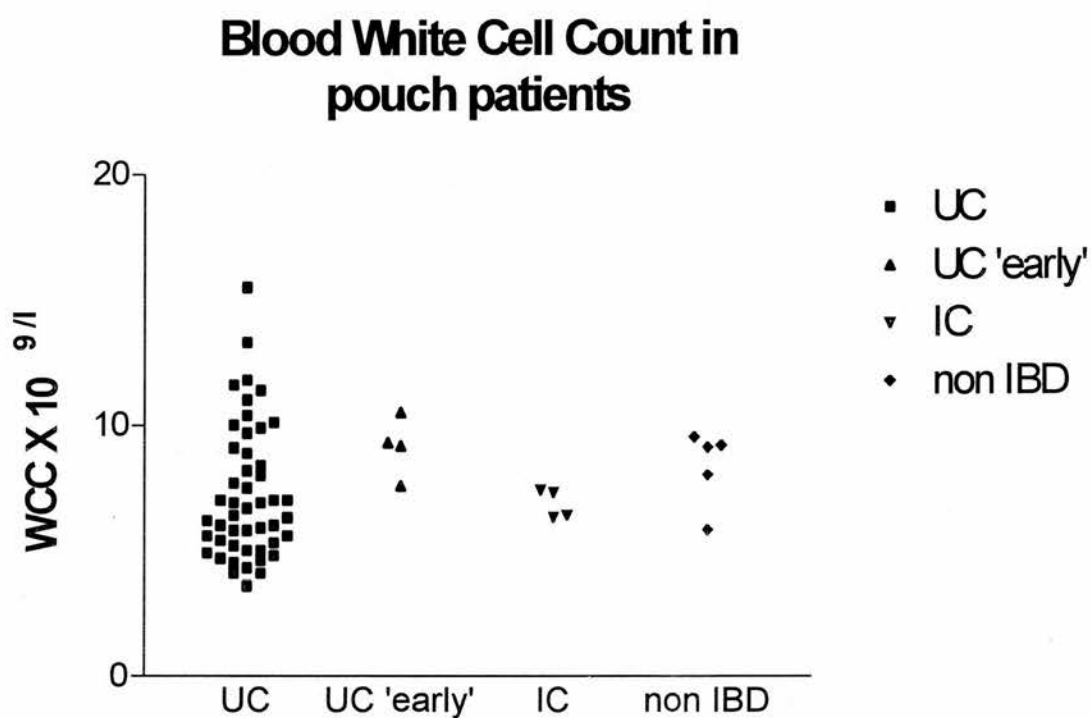
There was a statistically significant difference between the 'early' and the rest of the UC group ( $p=0.034$ ).

\*'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis

'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD



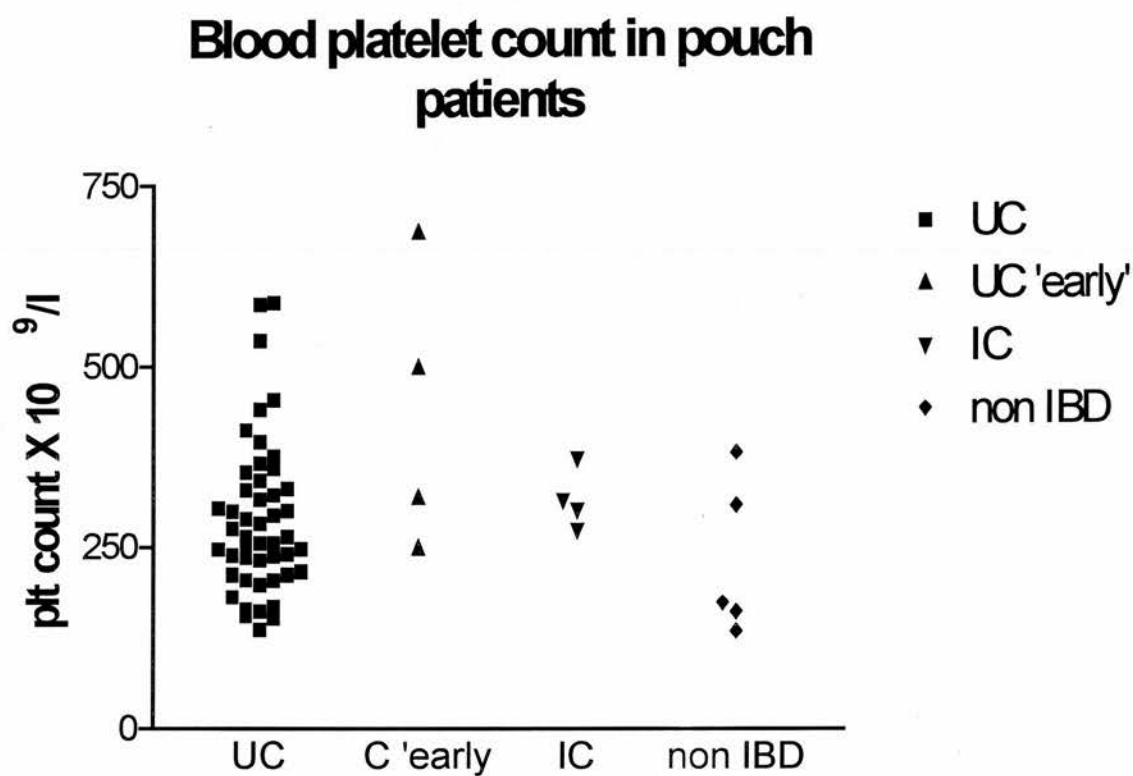
**Figure 4.7.** Estimation of systemic white cell count (WCC) in pouch patients' groups\*.

\*'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis

'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD



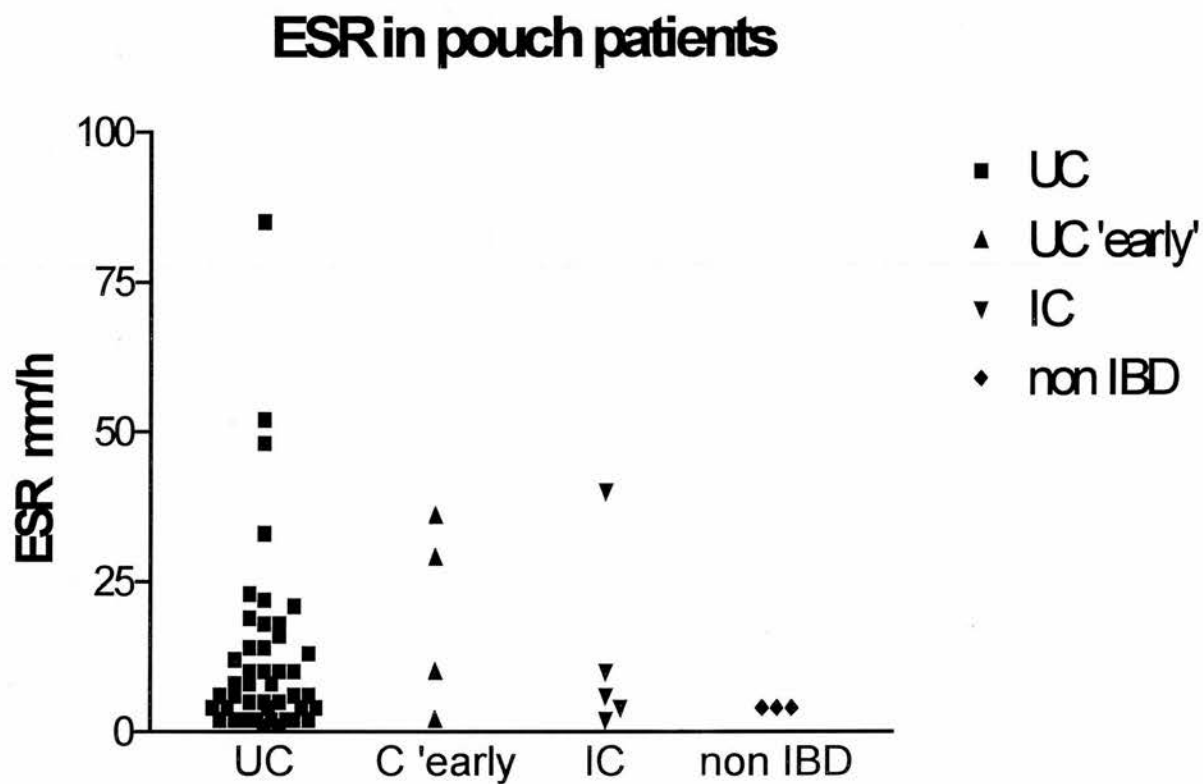
**Figure 4.8.** Estimations of systemic platelet (plt) counts in pouch patients' groups\*.

\*'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis

'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD



**Figure 4.9.** Estimation of Erythrocyte Sedimentation Rate (ESR) in pouch patients' groups\*.

\*'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis

'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD



#### **4.3.A.10c The Platelet Count**

The platelet counts of the groups examined are presented in Figure 4.8. In the UC group the platelet count ranged from  $137 \times 10^9/L$  to  $588 \times 10^9/L$  (median:  $278 \times 10^9/L$ ; 95%CI: 251, 308).

#### **4.3.A.10d The Erythrocyte Sedimentation Rate (ESR)**

The ESR measured in the UC group ranged from 1 to 85 mm/hr (median: 9 mm/hr; 95%CI: 6, 12). Female patients in the UC group had higher ESR compared to males ( $p=0.0174$ ). The ESR values for the UC patients, 'early', IC and 'non IBD' groups are presented in Figure 4.9.

#### **4.3.A.10e The C-Reactive Protein (CRP)**

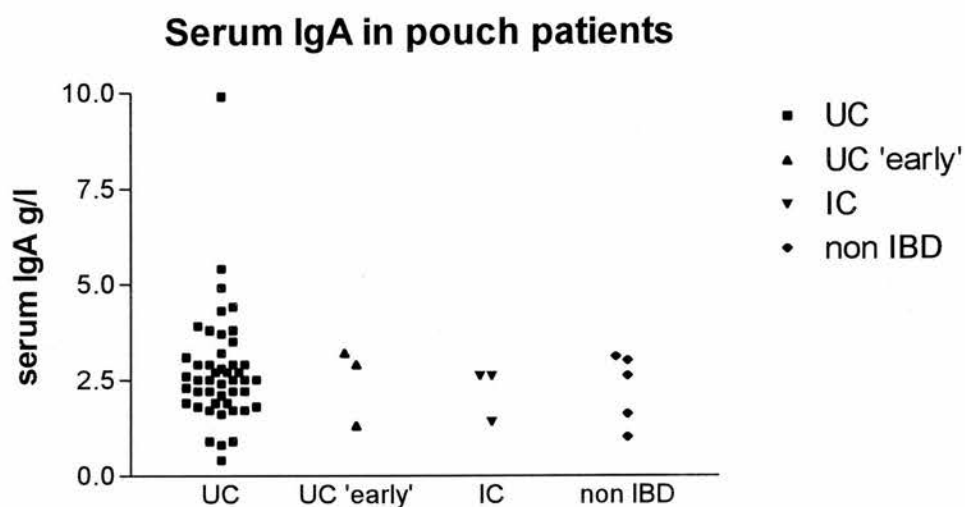
All groups, except for the 'early', had a median within the normal reference range for the laboratory (CRP: 1.5 mg/dL). Maximum value recorded in the UC group was 10.8 mg/dl. The CRP in the 'early' group was significantly higher compared to the UC group ( $p=0.0343$ ).

#### **4.3.A.10f Combination Of Systemic Inflammatory Markers**

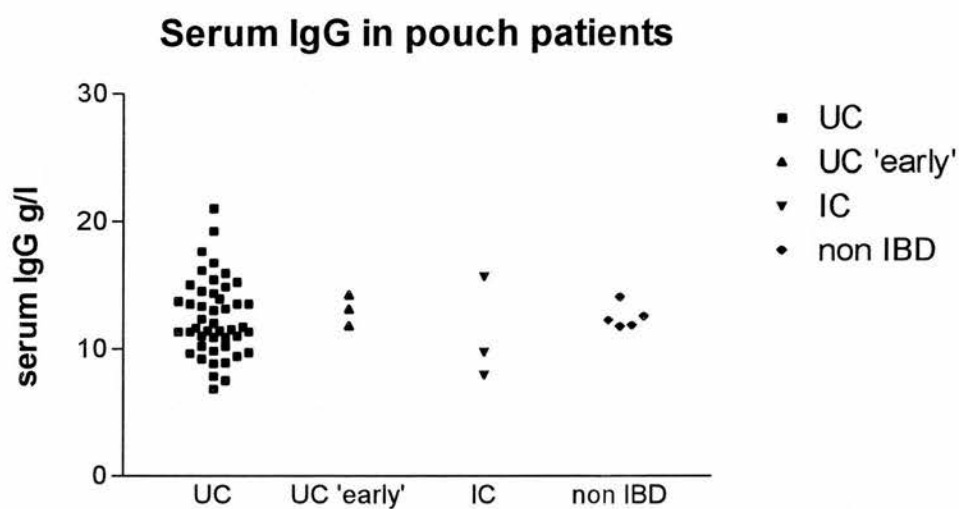
In the UC group, one patient with long-standing 'active pouchitis' had all four parameters high (WCC, Platelet count, ESR, CRP). Another patient with 'pouchitis' had all but the WCC raised. Three patients had high WCC and platelet count: one patient had 'pouchitis', one pyoderma gangrenosum, and the third one had a perianal abscess. Lastly, 6 patients with raised platelet count, 3 with raised platelet count and ESR and one with raised WCC had normal clinical parameters. One patient with raised ESR and CRP was on Metronidazole (200mg daily) and ASA.

#### **4.3.A.10g Serum Immunoglobulins**

Figures 4.10, 4.11, 4.12 demonstrate the serum IgA, IgG and IgM of the patients studied. The median values for the UC group were: IgA: 2.5 g/L



**Figure 4.10.** Serum Immunoglobulin-A (IgA) in pouch patients' groups\*.



**Figure 4.11.** Serum Immunoglobulin-G (IgG) in pouch patients' groups\*.

\*'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis;

'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months;

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis;

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD



**Table 4.19.** Anal cuff inflammation at endoscopy and pouch anal anastomosis' stenosis in the various pouch groups\*. The apparent differences are not statistically significant.

<b>Pouch groups</b>	<b>Anal cuff inflammation</b>	<b>Anastomotic stenosis</b>
UC group (n=56)	15	9
'early' group (n=5)	3	3
IC group (n=5)	0	2
non IBD group (n=5)	0	0

**Table 4.20.** The pouch biopsy neutrophilia and ulceration in the various pouch groups\*. There was no significant difference between the groups.

<b>Pouch groups</b>	<b>Mild neutrophilia</b>	<b>Severe neutrophilia</b>	<b>Ulceration</b>
UC group (n=56)	27	12	12
'early' group (n=5)	1	2	2
IC group (n=5)	2	1	1
non IBD group (n=5)	1	1	0

\*'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis;

'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months;

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis;

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD

(95%CI: 2.3, 2.9); IgG: 12.3 g/L (95%CI: 11.3, 13.2); IgM 1.2g/L (95%CI: 1.0, 1.4). These values correlated with each other, best correlation seen between IgA and IgG ( $r=0.40$ ,  $p=0.0053$ ).

### **4.3.A.11. Endoscopic Characteristics At Pouchoscopy**

#### **4.3.A.11a Endoscopic Score**

The presence of inflammatory stigmata was noted during endoscopy, with a severity score on a scale 1-6. Figure 4.13 shows the distribution of the scores in the various groups. In the UC group the median score was 2 (95%CI: 1.5, 2.5).

#### **4.3.A.11b Anal Cuff Inflammation & Pouch Anal Stenosis**

The incidence of anal cuff inflammation is presented in Table 4.19. This incidence in the UC group was 27%. The incidence of pouch anal stenosis was 16% in the UC group.

### **4.3.A.12. Pouch Biopsy Characteristics**

The results described in the following sections refer to the most severely affected pouch biopsies.

#### **4.3.A.12a Biopsy Neutrophilia & Ulceration**

Some degree of neutrophilia was present in 70% of the patients in the UC group. The presence of ulceration was noted in 24% of UC patients. See Table 4.20.

#### **4.3.A.12b Chronic Inflammation & Villous Atrophy**

Chronic inflammation of pouch biopsies was present in 70% of the UC cases (see Table 4.21). Villous atrophy was present in 28% of the UC patients (see Table 4.21).

**Table 4.21.** Chronic inflammation and villous atrophy in biopsies from the pouch mucosa in the various pouch groups\*. There was no significant difference between the groups.

<b>Pouch groups</b>	<b>Mild chronic inflammation</b>	<b>Severe chronic inflammation</b>	<b>Mild villous atrophy</b>	<b>Severe villous atrophy</b>
UC group (n=61)	27	12	11	5
'early' group (n=5)	2	1	2	0
IC group (n=5)	4	1	1	0
non IBD group (n=5)	4	0	1	0

**Table 4.22.** The number of previous episodes (none, one, several) of 'pouchitis like symptoms' as perceived by the pouch patients, presented in the various pouch groups\*.

<b>Pouch groups</b>	<b>None</b>	<b>One</b>	<b>Several</b>
UC group (n=56)	23	21	12
'early' group (n=5)	4	1	
IC group (n=5)	4		1
non IBD group (n=5)	5		

\*'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis

'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD



### **4.3.A.13. Pouchitis History & Disease Activity**

#### **4.3.A.13a Previous Episodes Of 'Pouchitis Like Symptoms'**

The number of previous episodes that could loosely be described as 'pouchitis' are given in Table 4.22. In total there was a history of such 'symptoms' in 59% of UC patients. This incidence is much higher than the incidence of 'pouchitis' as seen in literature (Sandborn, 1994). The difference however is that the above figure reflects the more subjective view of the patient's in regard to his own symptoms and has no objectivity of a medical assessment. The frequency of these episodes after the restoration of intestinal continuity was 0.3 per year (95%CI: 0.0, 0.5) in the UC group. However, the frequency of such episodes in UC patients with one previous episode was higher at 0.8 (95%CI: 0.4, 1.3;  $p < 0.05$ ).

#### **4.3.A.13b The Pouchitis Disease Activity Index (PDAI)**

Figure 4.14 shows the PDAI score in the patients' groups. Since this is a composite index of disease activity with clinical, endoscopic and histological features many of its determinants appear to correlate with its value as expected. In the UC group, 12 patients had PDAI greater or equal to 7, and could be named as having 'pouchitis', according to the PDAI definition.

#### **4.3.A.13c The Moskowitz Criteria Applied To The UC Group**

The description of pouchitis by Moskowitz requires diarrhoea more than 6 times per day, endoscopic stigmata of inflammation (greater than four), and histopathological evidence of ulceration and polymorphonuclear infiltration (greater than four). By Moskowitz definition, 7 out of 56 UC pouch patients appeared to have 'pouchitis'.

According to the PDAI, 12 patients were considered as having 'pouchitis'. This discrepancy between PDAI and Moskowitz criteria stems from the fact that in four out of five patients the endoscopy or biopsy was not severe enough to fulfill the Moskowitz criteria. This was the case despite the presence of severe



symptoms and especially urgency. The fifth patient clinically and endoscopically had very convincing pouchitis, but the biopsy result was essentially normal. This may have been influenced by concomitant treatment with metronidazole prior to the study.

If we 'define' the above seven patients as 'positive' for the Moskowitz criteria and the five patients who had high PDAI but did not fulfill the Moskowitz criteria as 'negative', the 'positive' group had more neutrophilia and ulceration in the biopsy ( $\chi^2=5.182$ ;  $p=0.023$ ). They had less urgency ( $\chi^2=8.4$ ;  $p=0.04$ ) and less evacuation difficulties ( $\chi^2=5.182$ ;  $p=0.023$ ). They also seem to have less history of pouch bleeding ( $\chi^2=4.286$ ;  $p=0.038$ ). It is thus clear that the emphasis in the 'negative' group was more on the symptoms and less on the biopsy results, which may explain the differences.

#### **4.3.A.14. The Clinical Diagnosis**

The diagnosis of 'pouchitis' or otherwise was made at each patient's interview depending on symptoms and endoscopic appearance (see Table 4.23). 19% of UC patients were described as having 'pouchitis'. This figure is within the expected rate of occurrence for pouchitis in the literature (Sandborn, 1994). A few patients seemed to have had other pouch problems (20%). These included: pouch anal stenosis (4%), severe anal cuff inflammation (3%), and perianal sepsis (1%).

A distinct group of patients had poor pouch function with no obvious evidence of pouch inflammation (12%). Two of these patients had been operated on for severe constipation. One of these is an epileptic on imipramine and the other one seems to have a psychosomatic behaviour problem. One patient in this group had ileal resection with corresponding short bowel syndrome. Another one was on antidepressants, before and after surgery. A third is again on antidepressants, but has an abnormally large pouch with deficient emptying. The fourth one has alcohol problems. The fifth patient, did not ever tolerate the medical treatment for IBD, only maintained on iron supplements. A month later after the study she developed a perianal abscess and

**Table 4.23.** The clinical categories at the time of the study, according to investigator's assessment.

<b>Pouch groups*</b>	<b>Normal</b>	<b>Pouchitis</b>	<b>Previous pouchitis</b>	<b>Other</b>
UC group (n=56)	27	7	11	11
'early' group (n=5)	1	3		1
IC group (n=5)	4	1		
non IBD group (n=5)	3			2

**Table 4.24.** The UC pouch patients with extraintestinal manifestations (EIM) and UC patients with no EIM. The PDAI score and WCC were higher in UC patients with EIM.

<b>UC pouch patients</b>	<b>with EIM (n=17)</b>	<b>no EIM (n=39)</b>	<b>p-value</b>
<b>PDAI</b>	5 (1-9)	3 (1-11)	0.0024
<b>WCC (X 10<sup>9</sup>/L)</b>	7.5 (4.1- 15.5)	5.8 (3.6- 11.8)	0.049

The results are expressed as median (range), the Mann-Whitney test is used for comparison.

\*'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis

'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD

later a pouch anal fistula. The sixth patient had endoscopic evidence of apparent mucosal prolapse.

If we compare the clinical diagnosis of 'pouchitis' with the diagnosis according to the Moskowitz criteria, then 4 patients were thought clinically to have pouchitis and were 'positive' for the Moskowitz criteria. Three more patients were thought clinically to have pouchitis (they also had high PDAI), but were 'negative' for the Moskowitz criteria.

## **4.3.B. Correlations Of Patients' Characteristics**

### **4.3.B.1. Medications Pre-Colectomy**

The percentage of routine colectomy was significant lower in the systemic steroid group (described in section 4.3.A.5.) compared to the other treatment subgroups ( $\chi^2 = 4.677$ ;  $p=0.031$ ). The complication rate was similar between these treatment subgroups even when they were stratified for urgency or the elective nature of the operation.

### **4.3.B.2. Extraintestinal Manifestations**

In the UC group there were similar characteristics in the functional and other parameters of the pouch, except for the blood WCC. This was higher in the patients who had a history of extraintestinal manifestations, though still within the reference range ( $p=0.049$ ). The PDAI score was also higher in these patients ( $p=0.0024$ ). Patients with 'pouchitis' according to the Moskowitz criteria had more extraintestinal manifestations ( $\chi^2=22.57$ ;  $p=0.0001$ ). Patients with a history of extraintestinal manifestations appeared to have more chronic inflammation manifested at their pouch biopsy ( $\chi^2=11.563$ ;  $p=0.001$ ). See Table 4.24.

### **4.3.B.3. The Histology Of Resected Colon Specimens**

#### **4.3.B.3.1. Histological Extent Of Disease**

In the UC group patients with pancolitis had significantly higher incidence of dysplasia in their resected specimens ( $\chi^2=5.122$ ,  $p=0.024$ ). All the other clinical features were similar in the two groups of left colitis or pancolitis. The incidence of pancolitis was not significantly different in patients with 'pouchitis' according to the Moskowitz criteria compared to the rest of the UC patients.

#### **4.3.B.3.2. Backwash Ileitis**

The clinical features were similar in those with backwash ileitis as compared to the rest of the UC patients. Patients with backwash ileitis however, described more 'pouchitis like symptoms', when interviewed ( $\chi^2=4.952$ ,  $p=0.026$ ,  $RR=3$ , 95%CI: 1.1, 7.8).

### **4.3.B.4 The Operative Characteristics**

#### **4.3.B.4.1. Number Of Urgent Operations & Operative Stages**

Patients who were operated on as emergencies did not seem to differ in their pre and postoperative characteristics. One difference emerging was that patients with evidence of villous atrophy in their pouch biopsy, had the colectomy performed as a routine ( $\chi^2=6.017$ ,  $p=0.014$ ,  $OR=5.2$ , 95%CI: 1.9, 13.8). Patients who had a single operation, appeared to have more villous atrophy in their pouches ( $\chi^2=5.204$ ,  $p=0.028$ ,  $OR=4$ , 95%CI: 1.16, 14).

#### **4.3.B.4.2. Pouch Design**

The endoscopic score, the incidence of villous atrophy and PDAI were higher in the 'J' group compared to the 'W' pouches in UC patients. These differences however could have been caused by the bias of patient selection in

each group. When the same parameters were examined in the pouches thought to be absolutely normal, indeed there was no difference. The 'J' group had more mucosal neutrophilia compared with the 'W' group ( $\chi^2=4.754$ ,  $p=0.029$ , OR=10.8, 95%CI: 1.2, 9.1). The functional score was comparable in the two pouch designs. See Table 4.25.

#### **4.3.B.4.3. Postoperative Complications**

Patients with a history of complications, had lower pouchoscopy score ( $p=0.0150$ ) and less villous atrophy ( $\chi^2=6.740$ ,  $p=0.01$ ). See Table 4.26.

#### **4.3.B.5. The 'Functional' Characteristics**

##### **4.3.B.5.1. The Bowel Activity**

In the UC group, the bowel activity during the day correlated to the bowel activity during the night ( $r=0.29$ ,  $p=0.0298$ ). The dose of metronidazole also correlated with the bowel activity during the day ( $r=0.36$ ,  $p=0.0071$ ), whereas the codeine dose related to the night bowel activity ( $r=0.33$ ,  $p=0.0052$ ). The dose of Loperamide also correlated with the night bowel activity ( $r=0.42$ ,  $p=0.0016$ ). Patients with previous 'pouchitis like symptoms' appeared to have more nocturnal evacuations ( $r=0.37$ ,  $p=0.0052$ ). Systemic parameters of inflammation, WCC, platelet count and ESR related to the night bowel activity ( $r=0.37$ ,  $p=0.0061$ ;  $r=0.39$ ,  $p=0.0061$ ; &  $r=0.44$ ,  $p=0.0029$  respectively).

##### **4.3.B.5.2. Pouch Related Difficulties:**

###### **Urgency - Difficult Evacuation - Perianal Discomfort - Social Handicap**

A. Patients with urgency had more evacuation difficulties ( $\chi^2=6.292$ ,  $p=0.012$ ) and more day and night soiling ( $\chi^2=7.418$ ,  $p=0.007$ ). These patients admitted to more social difficulties because of the pouch ( $\chi^2=7.418$ ,  $p=0.007$ ). In

**Table 4.25.** UC patients with 'J' and 'W' pouch and their platelet count. The platelet count was higher in the UC patients with 'J' pouch.

UC pouch patients	'J' pouch (n=21)	'W' pouch (n=5)	p-value
<b>Platelet count (X 10<sup>9</sup>/L)</b>	275 (153-535)*	199 (169-248)*	0.033

**Table 4.26.** The pouchoscopy score in UC patients with & without complications. The pouchoscopy score was higher in UC patients with no complications.

UC pouch patients	With complications (n=28)	No complications (n=28)	p-value
<b>pouchoscopy score</b>	2 (0-4)*	2 (0-6)*	0.015

**Table 4.27.** The ESR, PDAI and bowel activity in UC pouch patients with and without urgency. Patients with urgency had higher ESR, PDAI, bowel activity per day and night.

UC pouch patients	With urgency (n=14)	Without urgency (n=42)	p-value
<b>ESR</b>	21 (2-52)*	6 (1-85)*	0.0438
<b>PDAI</b>	6 (1-11)*	3 (1-9)*	0.0091
<b>Day bowel activity</b>	10 (7-15)*	6 (2-10)*	0.0001
<b>Night bowel activity</b>	2 (0-7)*	1 (0-5)*	0.0116

\*The results are expressed as median (range). Mann-Whitney test is used for comparison.

these patients the ESR and PDAI were higher ( $p=0.0438$  and  $p=0.0091$ , respectively). These patients had more bowel actions both during the day and night ( $p=0.0001$  and  $p=0.0116$ , respectively). See Table 4.27.

**B.** Patients with evacuation difficulties appeared to have more bleeding per pouch ( $\chi^2=13.71$ ,  $p=0.0000$ ; OR= 11.24, 95%CI: 3.1, 39.5).

**C.** Patients with perianal discomfort used more night pads ( $\chi^2=4.879$ ,  $p=0.027$ ), and changed their diet after surgery ( $\chi^2=4.424$ ,  $p=0.036$ ). The pouchoscopy score was higher ( $p=0.0297$ ). See Table 4.28.

**D.** Patients who changed their diet post surgery had a higher pouchoscopy score ( $p=0.0252$ ). See Table 4.29.

**E.** Patients who complained that the pouch interfered with their life, had a higher bowel activity both during the day and night ( $p=0.0196$  and  $p=0.0125$  respectively). These patients also reported more frequent 'pouchitis like symptoms' ( $p=0.0006$ ). The pouchoscopy score and PDAI were higher ( $p=0.0069$  and  $p=0.0001$ , respectively). See Table 4.30.

#### **4.3.B.5.3. Pouch Continence & Use Of Incontinence Pads**

The social functioning of patients was adversely affected by incontinence problems both during the day and night ( $\chi^2=7.405$ ,  $p=0.007$ ;  $\chi^2=10.567$ ,  $p=0.001$ , respectively). Patients who complained of day incontinence, were on higher doses of Metronidazole ( $p=0.0354$ ). See Table 4.31.

#### **4.3.B.5.4. Pouch Bleeding**

Patients with pouch bleeding had higher pouchoscopy score and PDAI ( $p=0.014$ ;  $p=0.003$ , respectively). See Table 4.32.



**Table 4.28.** The pouchoscopy score in UC pouch patients with & without perianal discomfort. This score is higher in UC patients with perianal discomfort.

UC pouch patients	Perianal discomfort (n=34)	No perianal discomfort (n=22)	p-value
pouchoscopy score	2 (0-6)*	1 (0-5)*	0.0297**

**Table 4.29.** The pouchoscopy score in UC pouch patients with and without diet change. The score is higher in the UC patients with change of diet.

UC pouch patients	With change of diet (n=22)	No change of diet (n=34)	p-value
pouchoscopy score	3 (0-6)*	2 (0-4)*	0.0252**

**Table 4.30.** The bowel activity, 'pouchitis like' symptoms, pouchoscopy score, PDAI and metronidazole dose in UC pouch patients with & without social handicap due to pouch. All these parameters were higher in UC patients with social handicap.

UC pouch patients	With social handicap (n=16)	No social handicap (n=40)	p-value
Day bowel activity	8 (2-15)*	1 (2-10)*	0.0196**
Night bowel activity	2 (0-5)*	1 (0-7)*	0.0125**
pouchitis like symptoms	3 (0-6)*	1 (0-6)*	0.0006**
pouchoscopy score	3 (0-5)*	2 (0-6)*	0.0069**
PDAI	7 (3-11)*	3 (1-9)*	0.0001**
Metronidazole mg/day	200 (0-1200)*	0 (0-600)*	0.0006**

\*The results are expressed as median (range). \*\* Mann-Whitney test is used for comparison.



**Table 4.31a.** Treatment with Metronidazole in UC pouch patients with and without incontinence. Patients with incontinence required higher doses of treatment.

UC pouch patients	With incontinence (n=12)	No incontinence (n=44)	p-value
Metronidazole mg/day	100 (0-1200)*	0 (0-600)*	0.0354**

**Table 4.31b.** The PDAI in UC pouch patients with and without night incontinence. The index is higher in UC patients with nocturnal incontinence.

UC pouch patients	With night incontinence (n=13)	No night incontinence (n=43)	p-value
PDAI	6 (1-11)*	3 (1-9)*	0.0030**

**Table 4.32.** The pouchoscopy score, PDAI, and serum IgM in UC pouch patients with and without bleeding per pouch. These parameters are higher in UC patients with bleeding per pouch.

UC pouch patients	Bleeding per pouch (n=16)	No pouch bleeding (n=40)	p-value
pouchoscopy score	3 (0-6)*	2 (0-4)*	0.0102**
PDAI	6 (2-11)*	3 (1-8)*	0.0030**
Serum IgM g/L	1.46 (0.27-2.80)*	1.08 (0.73-3.07)*	0.0093**

\* The results are expressed in median (range). \*\* Mann-Whitney test is used for comparison.

#### 4.3.B.5.5. Medications

The doses of codeine and loperamide correlated with the night bowel activity ( $r=0.33$ ,  $p=0.0151$ ;  $r=0.42$ ,  $p=0.0016$ , respectively).

Patients on metronidazole, as mentioned earlier, had more urgency ( $\chi^2=5.34$ ;  $p=0.021$ ), more evacuation difficulties ( $\chi^2=4.041$ ;  $p=0.045$ ), more day soiling ( $\chi^2=5.091$ ;  $p=0.0354$ ) and consequently worse social handicap ( $\chi^2=12.055$ ;  $p=0.001$ ). The dose of metronidazole correlated with the day bowel activity ( $r=0.36$ ,  $p=0.0071$ ), the pouchoscopy score ( $r=0.37$ ,  $p=0.0049$ ) and the number of previous 'pouchitis like episodes' reported by the patient ( $r=0.30$ ,  $p=0.025$ ). There was a positive correlation between the metronidazole dose and the CRP ( $r=0.63$ ,  $p<0.0001$ ).

#### 4.3.B.5.6. The 'Functional' score

The blood haemoglobin was inversely related to the 'functional' score, i.e. the worst the score the lowest the haemoglobin ( $r=0.45$ ,  $p=0.0013$ ). Platelet count, ESR and CRP correlated to the 'functional' score ( $r=0.32$   $p=0.024$ ;  $r=0.45$ ,  $p=0.0021$ ;  $r=0.43$ ,  $p=0.005$ , respectively). The 'functional' score correlated with the pouchoscopy score ( $r=0.40$ ,  $p=0.0019$ ). The medications codeine, loperamide, and metronidazole were also related to this functional score (best figure for metronidazole:  $r=0.46$ ,  $p=0.0004$ ). Patient's age at the time of pouch construction also seemed to influence the functional score ( $r=0.40$ ,  $p=0.0019$ ). The longer the time the pouch had been functioning, the better the functional score ( $r=0.30$ ,  $p=0.0206$ ).

### 4.3.B.6. Haematological Parameters

#### 4.3.B.6.1. Serum Immunoglobulins

ESR was relating both to serum IgG and IgM, ( $r=0.31$ ,  $p=0.0460$  and  $r=0.36$ ,  $p=0.0216$  respectively).

There was also a correlation between serum IgA and the age of colitis presentation and colectomy ( $r=0.31$ ,  $p=0.0335$ ). Serum IgG correlated with the number of previous episodes of 'pouchitis like symptoms' reported by the patient ( $r=0.31$ ,  $p=0.0387$ ).

Serum IgM was higher in patients who gave a history of pouch bleeding ( $p=0.0093$ ). See Table 4.32.

### **4.3.B.7. Endoscopic Characteristics At Pouchoscopy**

#### **4.3.B.7.1. The Endoscopic Score**

The pouchoscopy score correlated with the nocturnal bowel activity ( $r=0.36$ ,  $p=0.0071$ ) and the 'functional' score ( $r=0.40$ ,  $p=0.0019$ ). Patients who complained of perianal discomfort had a higher pouchoscopy score ( $p=0.0297$ ; see Table 4.28). Patients who had to change their diet or had a considerable social handicap also had a higher pouchoscopy score ( $p=0.0252$ ;  $p=0.0069$ , respectively; see Tables 4.29, 4.30). Patients who complained of pouch bleeding, had a higher score ( $p=0.014$ ; see Table 4.32). The 'J' pouch seemed to have more inflammation, but if we looked at the ones that were clinically normal pouches, there was no difference.

The pouchoscopy score related to the total WCC and the platelet count ( $r=0.32$ ,  $p=0.0244$  and  $r=0.29$ ,  $p=0.0487$  respectively). There was also correlation with CRP ( $r=0.45$ ,  $p=0.0031$ ).

The use of metronidazole was related again to the pouchoscopy score ( $r=0.37$ ,  $p=0.0348$ ). The number of previous episodes of 'pouchitis like symptoms' reported by the patient related to the pouchoscopy score ( $r=0.28$ ,  $p=0.0348$ ). Patients with villous atrophy appeared to have higher pouchoscopy scores ( $p=0.0435$ ). See Table 4.33.

### 4.3.B.7.2. Anal Cuff Inflammation & Pouch Anal Stenosis

Patients with anal cuff inflammation had higher pouchoscopy score ( $p=0.0113$ ) and complained more of perianal discomfort ( $\chi^2=5.034$ ,  $p=0.025$ ). See Table 4.34.

### 4.3.B.8. Pouch Biopsy Characteristics

#### 4.3.B.8.1. Biopsy Neutrophilia & Ulceration

Patients with biopsy neutrophilia had a higher incidence of family history of IBD ( $\chi^2=4.278$ ,  $p=0.031$ ; RR= 1.56; 95%CI: 1.04, 2.30). These patients had fewer complaints of urgency ( $\chi^2=4.561$ ,  $p=0.033$ ). Their pouch biopsy was characterized by high incidence of ulceration ( $\chi^2=7.88$ ,  $p=0.01$ ) and villous atrophy ( $\chi^2=5.439$ ,  $p=0.02$ ). Consequently patients with biopsy neutrophilia had a higher PDAI score ( $p=0.0221$ ). See Table 4.35a.

Patients with severe biopsy neutrophilia appeared to have some extra features. They tended to have more routine surgery ( $\chi^2=4.278$ ,  $p=0.039$ ), a higher WCC ( $p=0.0417$ ), higher serum IgG ( $p=0.027$ ) and IgM ( $p=0.0083$ ). These patients appeared to have modified diet more often ( $\chi^2=4.801$ ,  $p=0.029$ ). See Table 4.35b.

Patients with ulceration had more neutrophilia at their biopsy ( $\chi^2=6.109$ ,  $p=0.014$ ) and more villous atrophy ( $\chi^2=5.654$ ,  $p=0.018$ ).

#### 4.3.B.8.2. Chronic Inflammation & Villous Atrophy

A. Patients with chronic inflammation in the pouch biopsy had a history of extraintestinal manifestations some time in their life ( $\chi^2=35.073$ ,  $p=0.001$ ; RR= 1.8; 95%CI: 1.2, 2.5).

UC patients with severe inflammation had a higher platelet count ( $p=0.0290$ ), increased incidence of previous episodes of 'pouchitis like

**Table 4.33.** The pouchoscopy score in UC pouch patients with and without villous atrophy in pouch biopsy. The score is higher in UC patients without villous atrophy.

UC pouch patients	With villous atrophy (n=16)	No villous atrophy (n=40)	p-value
pouchoscopy score	2 (0-6)*	2 (1-6)*	0.018**

**Table 4.34.** The pouchoscopy score in UC pouch patients with and without anal cuff inflammation. The score is higher in patients with anal cuff inflammation.

UC pouch patients	anal cuff inflammation (n=16)	no anal cuff inflammation (n=40)	p-value
pouchoscopy score	2 (1-6)*	2 (0-6)*	0.0113**

\* The results are expressed as median (range). \*\* Mann-Whitney test is used for comparison.

**Table 4.35a.** The PDAI in UC pouch patients with and without neutrophilia in pouch biopsy. The index was higher in patients with pouch neutrophilia.

UC pouch patients	With neutrophilia (n=39)	No neutrophilia (n=17)	p-value
PDAI	4 (1-9)*	2 (1-11)*	0.0221**

**Table 4.35b.** The white cell count (WCC), serum IgG and IgM in UC pouch patients with and without severe neutrophilia in pouch biopsy. Patients with severe pouch neutrophilia had higher WCC and immunoglobulins.

UC pouch patients	severe neutrophilia (n=12)	minimal neutrophilia (n=34)	p-value
WCC in $\times 10^9/L$	8.2 (6.0-11.4)	5.9 (3.6-15.5)	0.0253
serum IgG g/L	13.60 (9.80-19.20)	11.35 (6.80-21.00)	0.0227
serum IgM g/L	1.35 (0.31-3.07)	1.08 (0.27-2.80)	0.0083

\* The results are expressed as median (range). \*\*Mann-Whitney test is used for comparison.

**Table 4.36.** The platelet count, ‘pouchitis like’ symptoms, pouchoscopy score, pouchitis disease activity index (PDAI) and the dose of metronidazole in UC pouch patients with and without severe chronic inflammation in pouch biopsy. The platelet count was higher in patients with severe chronic inflammation in pouch biopsy. These patients with severe chronic inflammation in pouch biopsy had a higher pouchoscopy score and PDAI. The dose of Metronidazole was significantly greater in patients with severe chronic inflammation as compared to those with mild chronic inflammation in pouch biopsy.

<b>UC pouch patients</b>	<b>Severe chronic inflammation (n=12)</b>	<b>Mild chronic inflammation (n=44)</b>	<b>p-value</b>
<b>Platelet count X10<sup>9</sup>/L</b>	331 (206-585)*	249 (137-588)*	0.0290**
<b>Pouchitis like symptoms</b>	1 (0-6)	1 (0-6)	0.0190
<b>Pouchoscopy score</b>	3 (1-6)	2 (0-6)	0.0220
<b>PDAI</b>	7 (1-9)	3 (1-11)	0.0036
<b>Metronidazole in mg/day</b>	100 (0-600)	0 (0-1200)	0.0030

\* The results are expressed as median (range). \*\*Mann-Whitney test is used for comparison.

symptoms' ( $p=0.0190$ ), worse pouchoscopy score ( $p=0.0022$ ) and were on higher doses of metronidazole ( $p=0.03$ ), had a higher PDAI score ( $p=0.0036$ ). These patients gave also a history of pouch bleeding ( $\chi^2=6.629$ ,  $p=0.01$ ) and admitted to a change in their diet ( $\chi^2=8.167$ ,  $p=0.004$ ). Their anal cuff was more commonly inflamed ( $\chi^2=4.197$ ,  $p=0.041$ ). In these patients there was no significant dysplasia reported in the resection specimen ( $\chi^2=4.006$ ,  $p=0.046$ ).

**B.** Patients with villous atrophy had mostly routine colectomies ( $\chi^2=6.077$ ,  $p=0.014$ ), with restoration performed at the same session as the colectomy ( $\chi^2=5.240$ ,  $p=0.022$ ), and accordingly with a lower total number of reported complications. They also seemed to belong mostly to the 'J' configuration pouch; the latter difference was not sustained when the pouches that were clinically normal were looked at separately. The biopsy neutrophilia in these patients with villous atrophy was higher ( $\chi^2=5.028$ ,  $p=0.025$ ) and their pouchoscopy score was worse ( $p=0.0435$ ). See Table 4.33.

### 4.3.B.9. Pouchitis History & Disease Activity

#### 4.3.B.9.1. The PDAI

The PDAI in UC patients correlated with the bowel activity during the day and during the night ( $r=0.39$ ,  $p=0.0033$  and  $r=0.42$ ,  $p=0.0016$  respectively). There was a correlation with the Öresland functional score ( $r=0.59$ ,  $p=0.0001$ ). Patients with urgency had a higher PDAI ( $p=0.0091$ ). Similarly patients with a history of night soiling had a higher index ( $p=0.0030$ ). Patients with pouch bleeding history also had a higher index ( $p=0.0006$ ). The 'J' pouches appeared initially to have higher scores; however the latter observation did not substantiate when only the absolutely normal 'J' or 'W' pouches were studied. The dose of metronidazole was related to the PDAI ( $r=0.39$ ,  $p=0.0023$ ). See Tables 4.27, 4.31b, 4.32.

The PDAI correlated with the ESR, CRP and serum IgM ( $r=0.36$ ,  $p=0.0138$ ;  $r=0.50$ ,  $p=0.0008$ ;  $r=0.32$ ,  $p=0.0264$ , respectively).



The endoscopic score correlated with the PDAI ( $r=0.81$ ,  $p<0.0001$ ). Patients with minimal neutrophilia in their pouch biopsy had lower PDAI ( $p=0.0221$ ). Moreover patients with severe chronic inflammation in the pouch biopsy had a higher PDAI score ( $p=0.0036$ ). See Tables 4.35a and 4.36.

#### **4.3.B.9.2. The Clinical Diagnosis**

Urgency in our series seemed to relate to 'other' problems ( $\chi^2=19.989$ ,  $p=0.001$ ; RR= 7; 95%CI: 2.7, 17.6) and less to active pouchitis. The recent onset of nocturnal soiling pointed towards active pouchitis ( $\chi^2=8.366$ ,  $p=0.04$ ; RR= 4.3; 95%CI: 1.1, 15.4). Another characteristic of active pouchitis appeared to be the change in diet that patient undertook to alleviate symptoms ( $\chi^2=8.464$ ,  $p=0.038$ ; RR= 10.2; 95%CI: 2.0, 50.0). This observation does not seem to be true for patients who were categorized as having 'other' problems. Patients who belong to this 'other' group complained more frequently of perianal discomfort ( $\chi^2=8.366$ ,  $p=0.04$ ; RR= 7.5; 95%CI: 1.5, 36) and were characterized by anastomotic stenosis ( $\chi^2=11.252$ ,  $p=0.013$ ; RR= 7.5; 95%CI: 1.5, 36).

## **4.4. DISCUSSION**

Patients with extraintestinal manifestations appear to have increased incidence of pouchitis (Lohmuller et al, 1990). In this study, patients with 'pouchitis' according to the Moskowitz criteria also had a higher incidence of extraintestinal manifestations. Furthermore, patients with extraintestinal manifestations had a higher PDAI score, a higher systemic WCC and more evidence of chronic inflammation in the pouch biopsy.

Backwash ileitis was not associated with pouchitis in our study. This is in agreement with previous reports (Gustavsson et al, 1987).

Patients who were operated for pancolitis did not appear to have higher incidence of pouchitis. This is in agreement with reports from Samarasekera and colleagues (1995), although Mikkola et al (1995) reported a higher incidence of

pouchitis in patients with previous pancolitis. In our study, patients with pancolitis had a higher incidence of dysplasia. This may be due to the bias of selection for colectomy. It may also be explained on the basis of more detailed histological analysis of specimens or as a true characteristic of IBD as seen in Scotland. Another hypothesis may be that this group represents patients with either more severe disease or disease of longer duration.

Patients who have been operated as routine and consequently had a single stage operation had a higher incidence of villous atrophy in their pouch biopsies. One hypothesis may be that patients had been on different immunomodulation treatment before surgery, which in turn may have affected epithelial kinetics. Indeed patients on high doses of steroids were hardly ever operated at a single stage, whereas those on combinations of 5-ASA and steroids or 5-ASA alone were very often operated routinely at one stage.

There were no differences in functional score between the two designs groups of 'J' and 'W'. The biopsy neutrophilia was higher in the 'J' pouch. This may be explained by either a stronger reaction to staples than to suture material used. On the other hand, the period of epithelial conduct with the antigenic faecal load may be different and thus may influence the mucosal neutrophilia? It is noticeable however, that one study has described a higher incidence of pouchitis in 'J' pouches (Hewett et al, 1995), whereas another study reported no difference in the evacuation parameters of either design (Heppel et al, 1987).

The overall complication rate of 52.4% is comparable to the one reported earlier (Fazio et al, 1995, Grotz & Pemberton, 1993).

The day and night bowel activity, as expected, related to each other and to the use of antidiarrhoeal medications. Urgency, since it is one of the constituents of the PDAI, not surprisingly related to this index. Furthermore the patients who complained of urgency seemed to exhibit more neutrophilia in their biopsy, and had a higher ESR. These patients also had increased bowel activity during day and night. If mucosal neutrophilia in pouches is associated with luminal neutrophilia, then urgency and diarrhoea are to be expected in pouches, whenever there is mucosal neutrophilia. This was shown in experiments by Madara et al who showed that luminal neutrophilia is a cause of diarrhoea (1991)

Pouchoscopy score related to the history of bleeding per pouch. This may signify the importance of the inclusion of bleeding in the calculation of the PDAI. The functional score was related to many parameters as expected, mainly to its own functional constituents and parameters of inflammation, systemic or endoscopic. This is probably due to the influence of bowel frequency onto score calculation, and the importance of this frequency as an indicator of pouch inflammation.

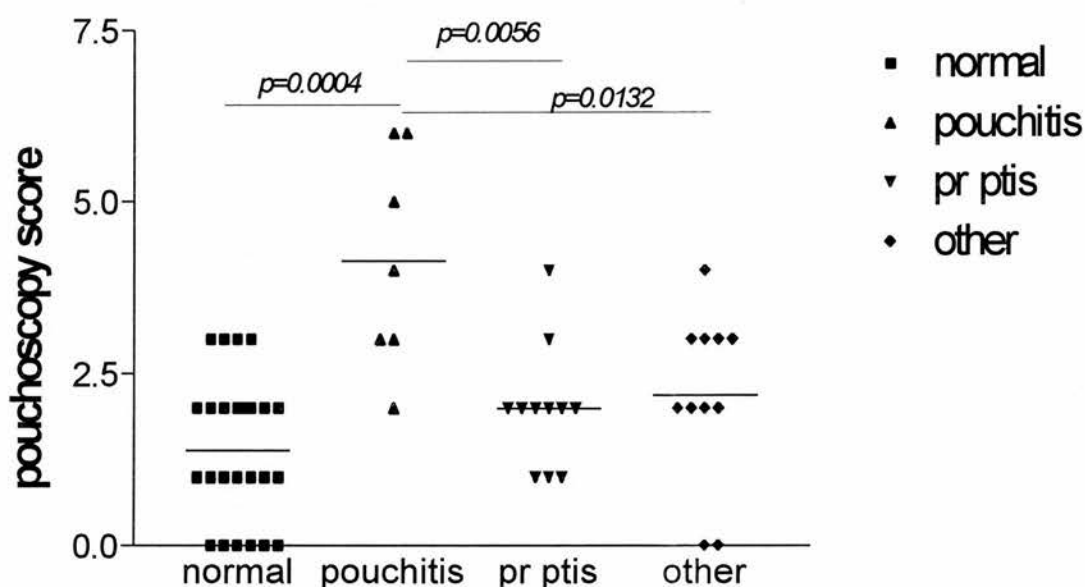
The pouchoscopy findings correlated with some of the systemic indices of inflammation: platelet count, WCC and CRP. This was not the case for the ESR or serum immunoglobulins. This once again indicates the difficulty in relating mucosal immune reactions and their histopathology to systemic immunity. It also discloses the need for a test to examine separately gut mucosa immune events. The endoscopic score was higher in patients on metronidazole; presumed treatment for pouchitis?. The association of the pouchoscopy score and villous atrophy may be explained since inflammation in the pouch mucosa, almost invariably involves a degree of villous atrophy. The observation that the endoscopic score was higher when there was anal cuff inflammation may signify that there is no real water-shed between anal cuff and pouch mucosa. In this case inflammation may spread from one area to the other. On the other hand, the amount of anal cuff left behind may at times inadvertently be much more in quantity than the operators estimate.

The histological characteristics, neutrophilia, ulceration and villous atrophy seemed to relate to each other. This may indicate that the immune cascade responsible diversified as to produce separate phenomena.

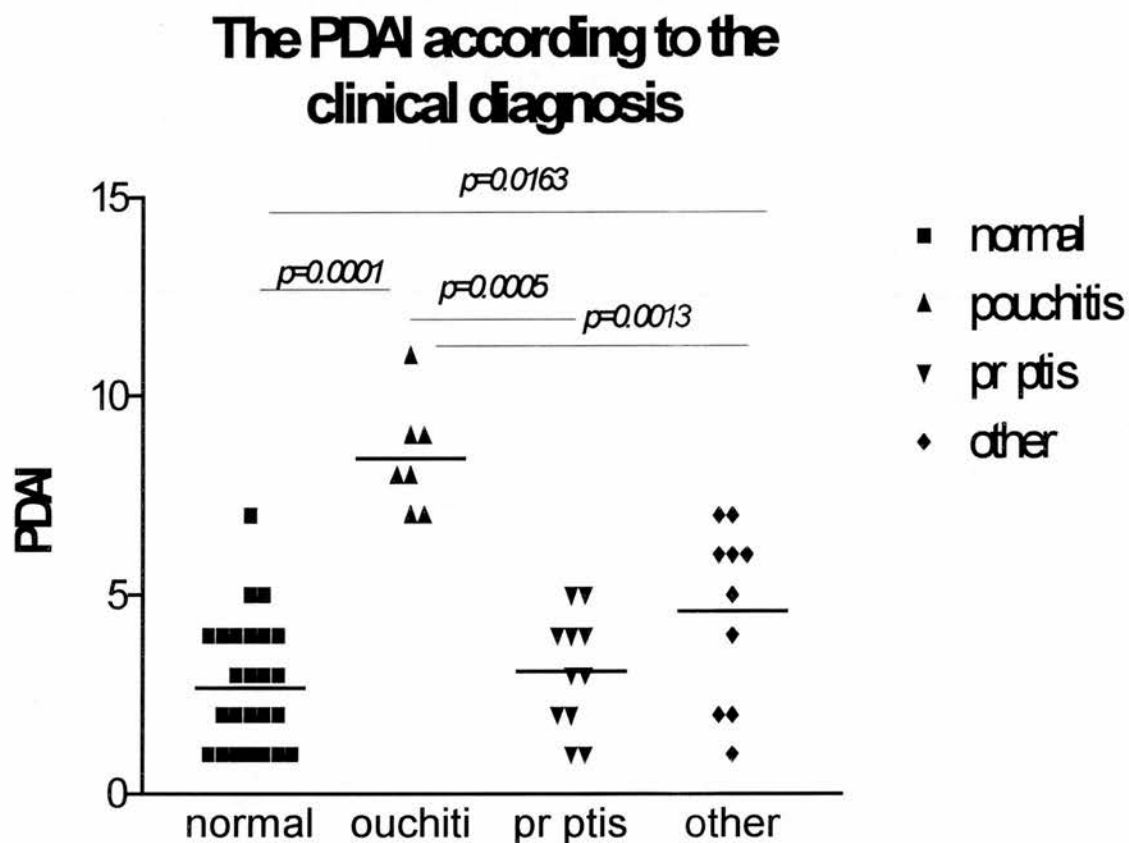
The calculation of the PDAI takes into consideration many parameters: clinical, endoscopic, histological. Accordingly the index associates with many of the functional parameters, the endoscopic and the histopathological ones.



### Pouchoscopy score according to clinical diagnosis



**Figure 4.16.** The pouchoscopy score in the various groups. These include the 'normal' group - UC patients with normal pouches; the 'pouchitis' group - UC patients with pouchitis according to the Investigator's diagnosis; the 'pr ptis' group - UC patients with previous episodes of pouchitis; and the 'other' group - UC patients with pouch anal stenosis, anal cuff inflammation, perineal sepsis and pouch dysfunction. The score was higher in the pouchitis group as compared to the 'normal', 'pr ptis' and 'other' groups.



**Figure 4.17.** The pouchitis disease activity index (PDAI) in the various groups. These include the ‘normal’ group - UC patients with normal pouches; the ‘pouchitis’ group - UC patients with pouchitis according to the Investigator’s diagnosis; the ‘pr ptis’ group - UC patients with previous episodes of pouchitis; and the ‘other’ group - UC patients with pouch anal stenosis, anal cuff inflammation, perineal sepsis and pouch dysfunction. The PDAI was higher in ‘pouchitis’ group as compared to the ‘normal’, ‘pr ptis’ and ‘other’ groups.

## Clinical Remarks: The Diagnosis of Pouchitis

Patients with ileoanal pouches share clinical features which are complex yet interrelated. It may be advantageous to the difficulties in establishing the diagnosis of pouchitis by using the information collected from the pouch patients.

The functional score, comprehensive as may be, only enables the separation of the normal pouches from the inflamed ones or the 'other' group (Figure 4.15). It cannot distinguish between the rest of the groups. For a more accurate assessment, endoscopic examination is needed, which will record inflammation, and obvious anastomotic stenosis (Figure 4.16). The use of the PDAI may be advantageous for the confirmation of the clinical status and a more objective means of comparing between patients (Figure 4.17).

The use of the routine systemic haematological parameters was not particularly helpful. Only the WCC and the platelet count were significantly higher in the acute pouchitis group compared to the normal group ( $p=0.0448$ ;  $p=0.0287$  respectively). ESR fluctuated irrespective of the clinical category.

It is already known that pouchitis is more common in patients with previous extraintestinal manifestations of IBD. This was also true in our series.

In conclusion, a previous history of **extraintestinal manifestations**, symptoms of **urgency**, **nocturnal soiling**, **recent change in diet**, **bleeding**, **perianal discomfort** along with the **endoscopic inflammatory appearances** were the most helpful clinical indices for the detection of the pouch pathology and thus the establishment of pouchitis in the outpatient setting. The addition of information from the pouch biopsy, especially the **presence of neutrophilia** aided the diagnosis. The PDAI is a useful tool for confirmation of the correctness of the clinical impression. It has to be said however, that in our study there was not always agreement between the PDAI and the Moskowitz criteria. This disagreement urges the development of a more objective test of inflammation, as it will be discussed in chapter VI.



# **CHAPTER V**

## **THE WHOLE GUT LAVAGE**

**in**

**- IBD**

**- ILEOSTOMY**

**- ILEOANAL POUCHES**



## **5.1. INTRODUCTION**

The whole gut lavage method has been used to categorise IBD patients according to their disease activity irrespective of the diagnosis of UC or CD. It is of interest to know however, whether the amount of substances retrieved in the WGLF is proportional to the length of the gut. One may expect that WGLF features may differ depending on the source if this is small bowel, large bowel or both. If either is true, then ileostomy patients may be perhaps expected to have lower levels of some of the substances measured in the lavage fluid. It is important to address the above mentioned questions before detailed analysis on the use of WGLF in ileoanal pouches is undertaken.

This chapter therefore, presents the study of whole gut lavage methodology in various groups of patients. This includes the study of patients with IBD and intact colon. Measurements obtained from these patients during whole gut lavage are compared to those obtained from patients with ileostomies. This will form the basis for the study of patients with ileoanal pouches using the whole gut lavage.

## **5.2. MATERIALS & METHODS**

### **5.2.1. IBD & Ileostomy Patients - IBD Database**

Data on the whole gut lavage procedure and analysis have been collected prospectively since 1991. This led to creation of a database in the GI laboratory at the Western General Hospital. The GI laboratory database allowed the identification of groups of IBD patients with intact colon and patients with ileostomies. These groups were fully characterised with both clinical and systemic parameters of inflammation. Table 5.1 describes the UC patients, whereas CD patients are described in Table 5.2. Ileostomy patients are presented in Table 5.3.

**Table 5.1** Demographic details of patients with ulcerative colitis and intact colon.

<b>Extent of Disease</b>	<b>M : F</b>	<b>Age*</b>
Pancolitis (n=17)	8: 9	35.5 (11-72) years
Distal colitis (n=24)	11:13	43.0 (23-83) years

**Table 5.2** Demographic details of patients with Crohn's disease and intact colon.

<b>Extent of Disease</b>	<b>M : F</b>	<b>Age*</b>
Oral disease (n=2)	1: 1	33.5 (22-45) years
Small bowel disease (n=36)	15:21	41.0 (13-77) years
Ileocolonic disease (n=11)	5: 6	21.0 (16-63) years
Colonic disease (n=25)	15:10	43.0 (21-78) years
Anorectal disease (n=25)	13:12	44.0 (10-90) years

**Table 5.3** Demographic details of patients with ileostomies.

<b>Disease</b>	<b>M : F</b>	<b>Age*</b>
Ulcerative Colitis (n=1)	0: 1	71 years
Crohn's Disease (n=21)	4:17	38 (21-77) years

\*The results are expressed in median (range). M:F male to female ratio.

### 5.2.2. The Pouch Patients

Sixty-six consecutive patients attending the General Surgical Departments of the Royal Infirmary and the Western General Hospital of Edinburgh were recruited for the study. Five patients were excluded as described in chapter III. Three more patients had to be excluded from the analysis, because the procedure followed did not comply fully with the protocol. In all three, although a gut lavage and pouchoscopy were performed, a biopsy was not taken because patients could not tolerate the procedure.

Consequently 66 patients were analysed. Male to female ratio was 37:29. Age ranged from 18.2 to 76.4 years (median: 40.7 years). The period of time between the study and restoration of intestinal continuity (i.e. closure of the ileostomy) ranged from 1.1 to 83.7 months postoperatively (median: 28.1 months). From the 66 patients, 61 patients had UC and 5 had IC. The characteristics of these groups are described in Table 5.4. The 'median time post pouch' is defined as the time between the gut lavage study and the restoration of intestinal continuity after pouch surgery. For our study the 61 UC patients and the 5 IC patients were combined in one IBD group and later compared with the ileostomy group.

### 5.2.3. Protocol & Assays Of WGLF

Bowel preparation with polyethylene glycol (PEG) electrolyte solution is commonly used prior to colonoscopy, barium enema or surgery. The immunological study of the bowel with the WGLF methodology is as follows: the patient fasts overnight, and then consumes the PEG solution at a steady rate. The rate of consumption is kept to one litre per hour adhering to the protocol as described by Choudari et al (1993).

It has been shown that after the passage of clear fluid, the concentration of immunoglobulins in the WGLF remains stable over time (Ferguson et al 1995). Because of the steady state thus achieved and for convenience, the first clear specimen obtained is processed immediately as described earlier in chapter III (see section 3.3.A.4. WGLF Processing).

**Table 5.4** Demographic details of the 66 pouch patients who participated in the study. There were no significant differences in age or sex between the groups (ulcerative colitis, indeterminate colitis).

<b>Pouch groups</b>	<b>M : F</b>	<b>Age*</b>	<b>Median time post pouch</b>
UC (n = 61)	36:25	43.0 (18.2-74.6) years	26.6 months
IC (n = 5)	1: 4	43.7 (35.7-61.0) years	26.5 months

**Table 5.5** WGLF parameters in IBD patients with intact colon (n=140)

<b>WGLF Parameter</b>	<b>Median (range)</b>
IgG	18 (0-781) $\mu\text{g/ml}$
Albumin	20.5 (0-470) $\mu\text{g/ml}$
$\alpha 1$ -Antitrypsin	8 (0-190) $\mu\text{g/ml}$
Haemoglobin	4.75 (0-106) $\mu\text{g/ml}$
Granulocyte Elastase	<0.039 (<0.039-2.742) $\mu\text{kat/l}$
IL-1 $\beta$	31.6 (0-4031) $\text{pg/ml}$
IL-8	6.15 (0-785.10) $\text{pg/ml}$

This protocol was approved by the Medicine Subcommittee of the Lothian's Area Ethics of Research Committee. The assays that are performed on the WGLF are described in detail in the appendix I, chapter III. In this chapter however, IL-8 is measured in filtered and processed samples. It has been shown that filtration gives lower values of detectable IL-8.

#### **5.2.4. Statistical Analysis**

The statistical analysis of the results used the median values and range; the non-parametric Mann-Whitney U test for unpaired comparison of observations; and the Kruskal-Wallis test for comparison of three groups. P values less than 0.05 were considered significant.

### **5.3. RESULTS**

#### **5.3.1. WGLF - IBD Patients With Intact Colon**

Whole gut lavage parameters measured in these patients included IgG, albumin,  $\alpha$ 1-antitrypsin, haemoglobin, neutrophil granulocyte elastase (GE) and the cytokines IL-1 $\beta$  and IL-8. Table 5.5 presents an overview of the values obtained in IBD patients with intact colon.

#### **5.3.2. WGLF - Ileostomy Patients**

Table 5.6 presents the values obtained in ileostomy patients. Two patients were excluded because of missing IgG result.

#### **5.3.3. WGLF - Pouch Patients**

Table 5.7 presents the WGLF parameters in the pouch patients recruited.

**Table 5.6** WGLF parameters in ileostomy patients (n=20)

<b>WGLF Parameters</b>	<b>Median (range)</b>
IgG	6 (0-117) µg/ml
Albumin	13 (0-140) µg/ml
α1- antitrypsin	4.5 (0- 24) µg/ml
Haemoglobin	2.8 (0- 9) µg/ml
Granulocyte elastase	<0.039 (<0.039- 0.1500) µkat/l
IL-1β	0.6 (0.0- 228.2) pg/ml
IL-8	15.4 (0.0- 116.4) pg/ml

**Table 5.7** WGLF parameters in IBD ileoanal pouch patients (n=66)

<b>WGLF Parameters</b>	<b>Median (range)</b>
IgG	4 (1 - 69) µg/ml
Albumin	9 (1 - 71) µg/ml
α1- antitrypsin	4.5 (1 - 44) µg/ml
Haemoglobin	3 (0.4-199) µg/ml
Granulocyte elastase	<0.039 (<0.039- 0.5110) µkat/l
IL-1β	6.56 (0.0-143.15) pg/ml
IL-8	17.95 (0.0-164.52) pg/ml

### 5.3.4. Comparisons Between Patients' Groups

Studies performed in the Gastrointestinal laboratory showed that the level of WGLF IgG  $>10\mu\text{g/ml}$  characterises active IBD (Brydon et al, 1993). This appears to be a more objective means of distinction between active and inactive disease (Acciuffi et al, 1996). Accordingly the patients with IBD and intact colon, patients with ileostomy and patients with pouch were separated into two groups of 'active' (WGLF IgG  $\geq 10\mu\text{g/ml}$ ) or 'inactive' disease (WGLF IgG  $<10\mu\text{g/ml}$ ). The groups of 'active' disease were compared with the groups of 'inactive' disease as the Latin Square principle dictates.

All parameters (IgG, albumin,  $\alpha 1$ -antitrypsin, haemoglobin, IL-1 $\beta$ , IL-8 and GE) were significantly higher in 'active' disease as compared to the 'inactive' disease. WGLF haemoglobin and GE measured in ileostomy patients however, were not different in 'active' compared to 'inactive' disease groups.

#### 5.3.4.1. Inactive Disease: WGLF IgG $< 10\mu\text{g/ml}$

Table 5.8 presents the values and the comparisons between IBD patients with intact colon (IBD,  $n=49$ ), ileostomy patients ( $n=12$ ) and with pouch patients ( $n=49$ ). All patients had 'inactive' disease with WGLF IgG  $<10\mu\text{g/ml}$ .

WGLF albumin was lower in the ileostomy patients compared to the pouch and IBD with intact colon (see Figure 5.1). WGLF IL-8 however, was lower in patients with intact colon compared with ileostomy and pouch patients devoid of colon (see Figure 5.2).

#### 5.3.4.2. Active Disease: WGLF IgG $\geq 10\mu\text{g/ml}$

Table 5.9 describes the values obtained in active disease in IBD patients with intact colon ( $n=92$ ), ileostomy patients ( $n=8$ ), and pouch patients ( $n=17$ ). All patients had WGLF IgG  $\geq 10\mu\text{g/ml}$ .

WGLF IgG and  $\alpha 1$ -antitrypsin were higher in IBD patients with intact colon (see Figures 5.3 and 5.4). There was no difference in GE between pouch and ileostomy. However, when pouch patients on metronidazole were excluded,

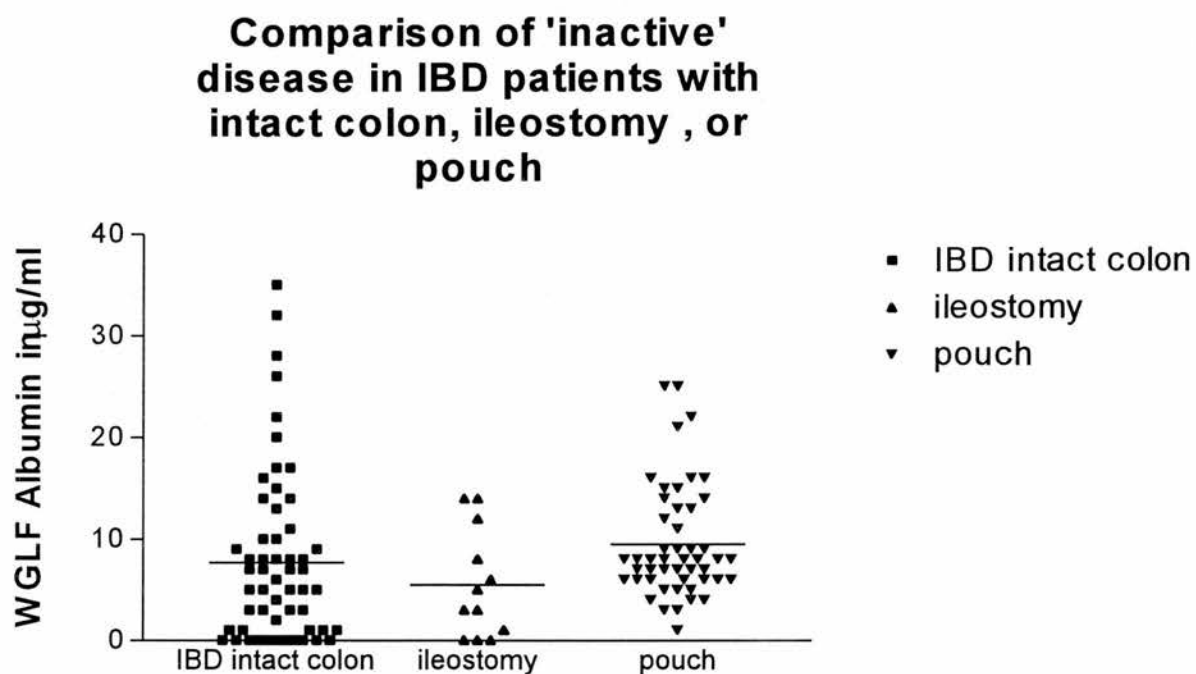
**Table 5.8** The WGLF parameters in the various groups of patients, all with inactive disease (WGLF IgG<10µg/ml). The albumin and IL-1β were higher in the pouch group as compared with the ileostomy group. The haemoglobin was lower, whereas IL-8 was higher in the pouch group as compared with the IBD group.

<b>WGLF parameter</b>	<b>IBD intact colon (n=49)</b>	<b>Ileostomy (n=12)</b>	<b>Pouch (n=49)</b>	<b>p value</b>
IgG µg/ml	4 (1-9)	3 (0-6)	3 (1-8)	
Albumin µg/ml	7 (0-26)	4 (0-14)*	8 (1-25)*	0.0183
AlAT µg/ml	3 (0-18)	1 (0-10)	3 (1-15)	
Hb µg/ml	3.9 (1-17.8)*	2.8 (1-4)	2 (0.4-19.9)*	0.0039
GE µkat/l	<0.039 (0.039-1.368)	<0.039 (0.039- 0.055)	<0.039 (0.039-0.079)	
IL-1β pg/ml	2.6 (0-931)	0.0 (0-17)*	4.2 (0-143.15)*	0.0157
IL-8 pg/ml	0.6 (0-14.4)*	7.5 (0.0-15.4)	5.9 (0.0- 42.8)*	0.0001

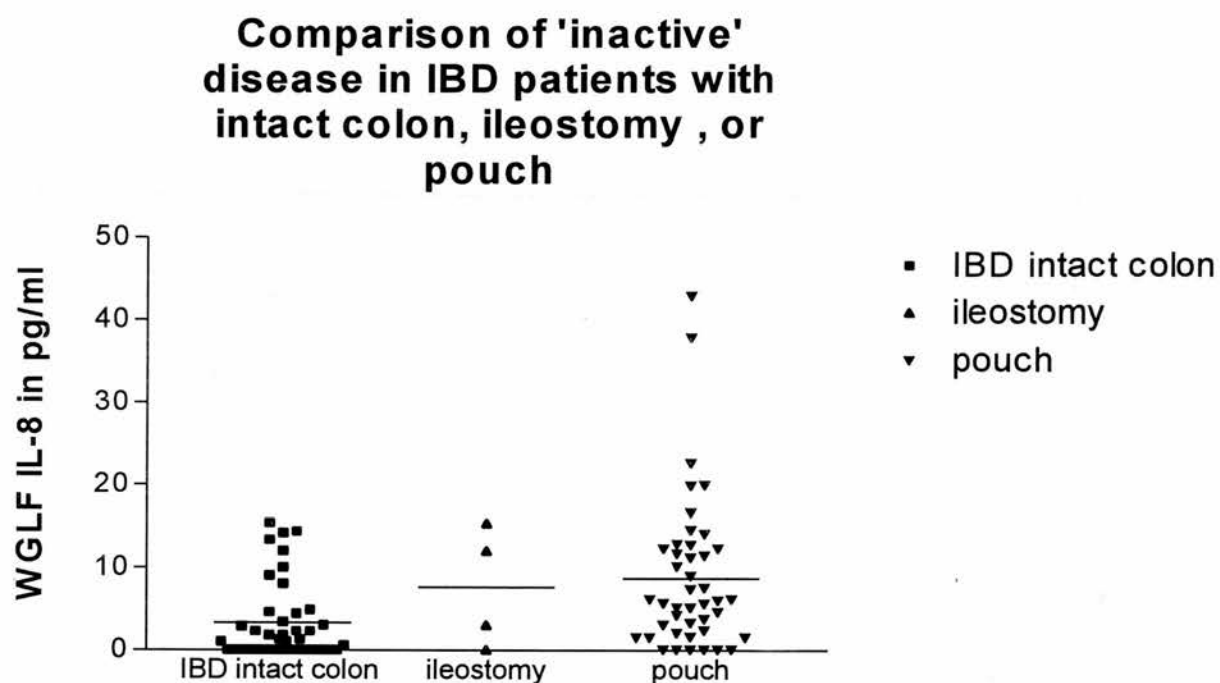
The results are presented in median (range). Mann-Whitney test was used for comparison of groups of patients.

‘\*’ indicate significant results between the groups





**Figure 5.1** The WGLF albumin in IBD patients with intact colon, ileostomy, or ileoanal pouch with inactive disease (WGLF IgG <  $10\mu\text{g/ml}$ ). Highest values observed in pouch patients. Transverse lines represent the mean values.



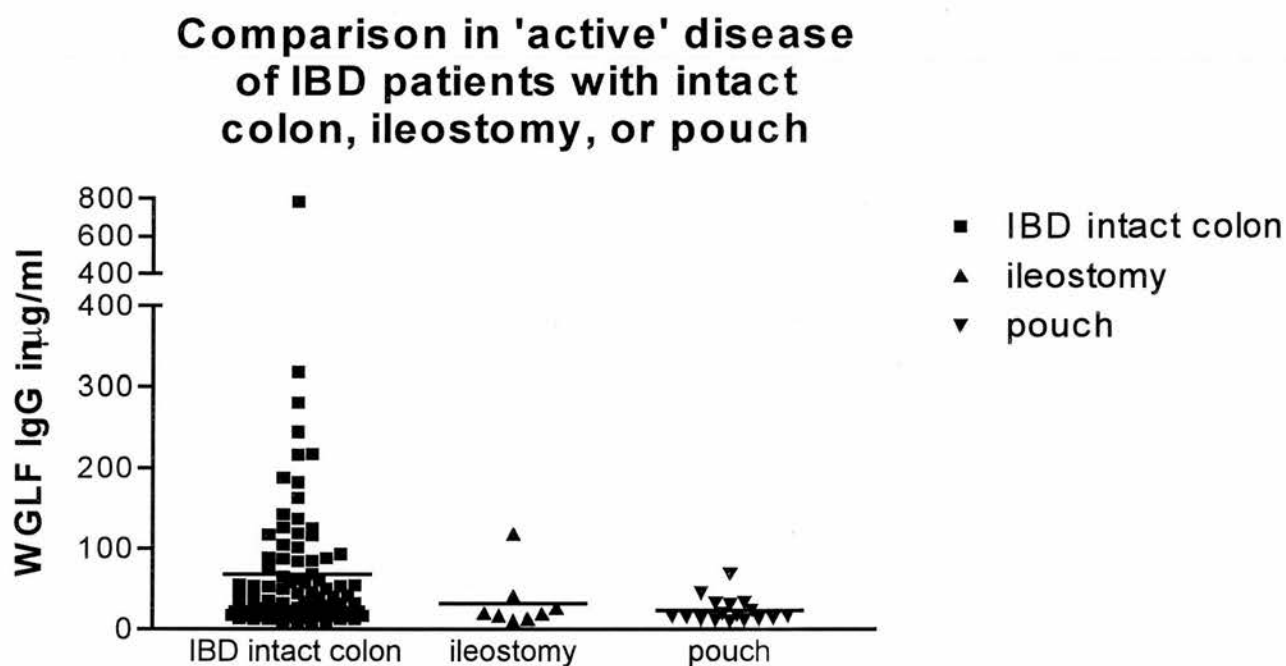
**Figure 5.2.** The WGLF IL-8 in IBD patients with intact colon, ileostomy, or pouch with inactive disease (WGLF IgG<10 $\mu$ g/ml). The WGLF IL-8 was higher in patients with pouch and ileostomy. Transverse lines represent the mean values.

**Table 5.9** The WGLF parameters in the various groups of patients with active disease (WGLF IgG $\geq$ 10 $\mu$ g/ml). The WGLF IgG, A1AT, IL-1 $\beta$  were higher in the IBD group as compared with the pouch group; the A1AT and Hb were higher in the IBD group as compared to the ileostomy group; WGLF Hb was also higher in the pouch as compared to the ileostomy group.

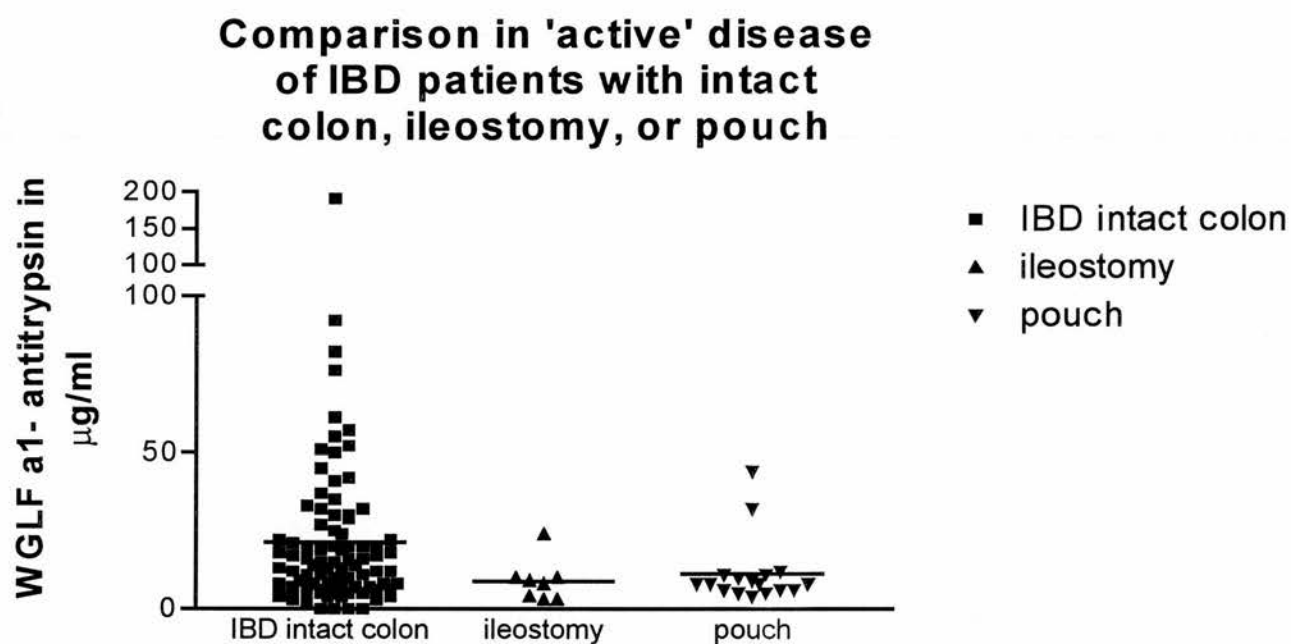
<b>WGLF parameter</b>	<b>IBD intact colon (n=92)</b>	<b>Ileostomy (n=8)</b>	<b>Pouch (n=17)</b>	<b>p value</b>
IgG $\mu$ g/ml	39.5 (10-781)*	18.5(10-117)	17(11-69)*	*0.0019
Albumin $\mu$ g/ml	46(2-470)	66(15-140)	29(12-71)	
A1AT $\mu$ g/ml	17(0-190)* <sup>+</sup>	8.5(3-24) <sup>+</sup>	8(4-44)*	<sup>+</sup> 0.0254 *0.0126
Hb $\mu$ g/ml	6.2(0-58)*	2.5(0-9)* <sup>+</sup>	5(2-156) <sup>+</sup>	*0.0289 <sup>+</sup> 0.0343
GE $\mu$ kat/l	0.104 (0.039-2.742)	<0.039 (undetectable)	0.081 (0.039-0.511)	
IL-1 $\beta$ pg/ml	119.6 (0.0-4031)*	106.6 (10-228.2)	44.9 (8.8-137.4)*	*0.0391
IL-8 pg/ml	10.6 (0.0-310)	51.5 (15.4-116.4)	36.9 (0.0-164.5)	

The results are presented as median (range). The Mann-Whitney test was used for comparison.

‘\*’, ‘<sup>+</sup>’ represent the significant results and their p value



**Figure 5.3.** The WGLF IgG in IBD patients with intact colon, ileostomy and pouch; all with active disease (WGLF IgG  $\geq 10 \mu\text{g/ml}$ ) Highest values are observed in IBD intact colon patients. Transverse lines represent the mean values.



**Figure 5.4.** The WGLF A1AT in IBD patients with intact colon, ileostomy and pouch; all with active disease (WGLF IgG  $\geq 10 \mu\text{g/ml}$ ). Highest values are observed in IBD intact colon patients. Transverse lines represent the mean values.

GE in pouch patients appeared to be higher than in ileostomy patients ( $p=0.0412$ ).

## 5.5. DISCUSSION

The WGLF methodology has been used in IBD to categorise patients according to the disease activity as expressed by the gut protein loss of WGLF IgG (Choudari et al 1993). A cut off point of  $10\mu\text{g/ml}$  is used to separate active from inactive IBD. This cut off point was used in this study for all groups examined: IBD intact colon, ileostomy and pouch.

All the WGLF parameters differ in active compared with inactive disease, both in IBD patients and in pouch patients. Similar results were obtained for the ileostomy patients, except for haemoglobin and GE. This study shows that WGLF methodology may be readily applicable in patients with ileoanal pouches and ileostomies. Furthermore, the levels of substances measured in ileoanal pouches and ileostomies do not differ from those measured in patients with intact colon.

In active disease, as defined by WGLF IgG  $\geq 10\mu\text{g/ml}$ , the WGLF IgG and  $\alpha 1$ -antitrypsin were higher in patients with intact colon. This may indicate that the phenomenon of protein loss in active inflammation may be different in the presence of intact colon compared to those devoid anatomically of colon. Albumin, however, is not different in the various groups. This may be due to the fact that albumin may decay more readily in whole gut lavage samples (Gaspari et al, 1988).

In inactive disease, albumin was lower in ileostomies, with similar levels between patients with intact colon or pouches. Albumin in ileoanal pouches is high. This may indicate that the pouch function as a capacitance organ influences the observed protein loss in pouches. On the other hand the levels of IL-8 were equally high both in ileostomies and in pouches; this may indicate that the mechanisms of luminal cytokine release or even of luminal chemotaxis for neutrophils differ between colon and small bowel. Differences in luminal

neutrophilia between small bowel and colon have already been described (Handy et al, 1996). Neutrophilia is seen mainly in active UC and Crohn's colitis but not in active small bowel CD.



# **CHAPTER VI**

**WHOLE GUT LAVAGE**

**in**

**ILEOANAL POUCHES**



## 6.1. INTRODUCTION

The use of whole gut lavage to obtain information about immunological aspects of UC patients and ileostomies has been explored earlier (see Chapters III & V). Studies in inflammatory bowel disease (IBD) showed that disease activity in IBD may be associated with gut protein loss (O'Mahony et al, 1991). The WGLF methodology allows measurement of immunoglobulins such as IgG (see Chapter III). The WGLF IgG provides an objective and accurate quantification of disease activity in IBD (Choudari et al, 1993). The cut off point for WGLF IgG of 10µg/ml is used by the GI laboratory (WGH) to identify 'activity' in IBD (Brydon et al, 1993). On the other hand, approximately 43% of IBD patients studied with WGLF method appear to have gut blood loss (Ferguson et al, 1996).

The pro-inflammatory mediators such as IL-1 $\alpha$  and IL-1 $\beta$  are of particular interest in IBD. IL-1 $\beta$  appeared to be elevated in mucosa of endoscopic biopsies, correlating with disease activity (Brynskov et al, 1992). The interleukin IL-8 is a potent chemoattractor and activator of neutrophils, which can cause mucosal destruction. WGLF studies on IL-1 $\beta$  and IL-8 showed that these proinflammatory cytokines are higher in active IBD (Ghosh, 1995). Furthermore, neutrophil polymorphs are a prominent feature of the intestinal inflammatory infiltrate in IBD (Lobos et al, 1987). Cytology of the WGLF led to the study of neutrophil migration to the gut lumen (Handy et al, 1995). Luminal neutrophilia characterised active colonic Crohn's and UC. Thus, the neutrophil granulocyte elastase (GE) measurement in the WGLF was utilised to assess luminal neutrophilia in IBD (Handy et al, 1996). Total immunoglobulins were assessed in WGLF: total IgA was higher in UC patients and IgA antibodies to bacteroides were higher in Crohn's disease (Srivastava et al, 1991; Poxton et al, 1995; respectively).

Pouchitis is relatively common in UC accounting up to 30% of the patients (Fozard and Pemberton, 1992). Many common elements in the natural history, pathology, response to treatment and complications between pouchitis and IBD led to the hypothesis that pouchitis may be considered as a form of IBD (Banerjee,

1994). Thus, the WGLF method as described earlier (see Chapter III) along with pouch assessment including clinical, endoscopic, and histological criteria have been combined in an attempt to characterise ileoanal pouches in health and during pouchitis.

In this chapter the parameters studied in the WGLF are presented, both in the main UC group of pouches and in the other diagnostic groups. Possible correlations have been sought amongst the parameters studied, but also to the range of clinical entities described already in Chapter IV.

The parameters studied in WGLF are presented in five main groups:

- A. Gut protein loss (IgG, albumin,  $\alpha$ 1-antitrypsin)
- B. Gut blood loss (haemoglobin)
- C. Cytokine profile (IL-1 $\beta$ , IL-8)
- D. Luminal neutrophil migration (neutrophil granulocyte elastase)
- E. Immunoglobulins (total IgA & IgM)

## **6.2. PATIENTS & METHODS**

### **6.2.1a. Patients**

Seventy-four consecutive patients attending the General Surgical Departments of the Royal Infirmary and the Western General Hospital of Edinburgh were recruited for the study. Five patients were excluded as described in Chapter III. Three more patients had to be excluded from the analysis, because the procedure followed did not comply fully with the protocol. Consequently 71 patients were analyzed.

Male to female ratio was 40:31. Age ranged from 18.2 to 76.4 years (median: 41.7 years). The period of time between the study and restoration of intestinal continuity (i.e. closure of the ileostomy) ranged from 1.1 to 83.7 months (median: 26.9 months) postoperatively.

**Table 6.1.** Demographic details of the 71 pouch patients who participated in the study. There were no significant differences in age or sex between groups (UC, 'early' UC group, 'non IBD', and IC).

<b>Pouch groups</b>	<b>M : F</b>	<b>Age (years)</b>	<b>Median time post pouch</b>
UC (n = 56)	32:24	42.8 (18.2-74.6)	28.9 months
UC 'early' (n=5)	4:1	36.3 (23.0-35.0)	3.4 months
IC (n = 5)	1:4	43.7 (35.7-61.0)	26.5 months
'non IBD' (n = 5)	3:2	48.2 (23.2-76.8)	25.6 months

'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis

'early UC group' - patients who underwent restorative proctocolectomy for UC within the last 6 months

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis

'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD

The results are expressed as median (range)

M:F= male to female ratio

## **6.2.1b. Patient Subgroups**

Patients were subdivided according to the original diagnosis for which the pouch was created. Sixty-one patients had UC, 5 had indeterminate colitis (IC), and 5 patients had other diseases: refractory constipation, familial adenomatous polyposis coli (FAP) or multiple synchronous cancers (non IBD group; see Chapter III, section 3.2.3.4)

Another subdivision became apparent in the early stages of the study. There were several patients who had recently had their pouch constructed for UC. A cut off point of six months was taken to separate this 'early' group, which corresponded to the differences in the clinical profile observed for these patients. This group numbered five patients (Chapter III, section 3.2.3.2).

The characteristics of these groups are described in Table 6.1. The 'non IBD' group included 2 patients who had their colectomy for multiple synchronous cancers, one FAP patient, and two who had their colectomy for intractable constipation, after failure of ileorectal anastomosis. The term 'median time post pouch' is the time between my study and when the patient had the intestinal continuity restored after his/her pouch surgery.

## **6.2.2. Methods**

### **6.2.2.1. Introduction**

Detailed analysis of the method followed is given in Chapter III. The patients were seen first in the outpatient department, except from the inpatients, and the procedure was explained to them. If they agreed, they were called for the investigation on another day.

The study took place in the investigation room of the Gastrointestinal Laboratory at the Western General Hospital or on ward 39 and the attached endoscopy suite at the Centre of Liver and Digestive Disorders (CLDD) at the

Royal Infirmary of Edinburgh. The inpatients were studied in the corresponding Surgical wards of the two Hospitals (Surgical South and Wards 11 and 12).

The protocol followed included:

- A. Whole gut lavage
- B. History taking
- C. Blood tests
- D. Pouchoscopy and biopsy

#### **6.2.2.2. The Whole Gut Lavage**

Patients after overnight fasting consumed the polyethylene glycol electrolyte (PEG) solution at a steady rate (one litre per hour), adhering to the protocol (Choudari et al, 1993). After the passage of fluid stools, it has been shown that the concentration of immunoglobulins in the WGLF remains stable over time (Ferguson et al 1995). Because of the steady state thus achieved and for convenience, the first clear specimen obtained was processed immediately the way it is described earlier (see Chapter III; section 3.3.A.4.2: Preparation & handling of WGLF). The assays performed are described in detail in appendix I in Chapter III.

#### **6.2.2.3. The Clinical Investigations**

In brief, the patients were attending for whole gut lavage study of their pouch and at the same time their medical history was obtained with the help of a pre-structured questionnaire (see appendix III, Chapter III). Blood tests were performed for full blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum immunoglobulins (IgG, IgM, IgA). Following that, pouchoscopy and biopsy were performed, as described in Chapter III, section 3.6.

#### **6.2.2.4. Statistical Analysis**

The statistical analysis of the results used the median values; 95% confidence interval (CI), interquartile (IQ) or range; the Pearson correlation coefficient and its p value; the non-parametric Mann-Whitney U test for unpaired comparison of observations;  $\chi^2$  with Yates correction with Odds Ratio (OR) or Relative Risk (RR). P values less than 0.05 were considered significant.

### **6.3. RESULTS**

#### **6.3.A. Gut Protein Loss In Ileoanal Pouches**

##### **6.3.A.1. WGLF Immunoglobulin G (WGLF IgG)**

###### **6.3.A.1.1. WGLF IgG In The Various Diagnostic Groups**

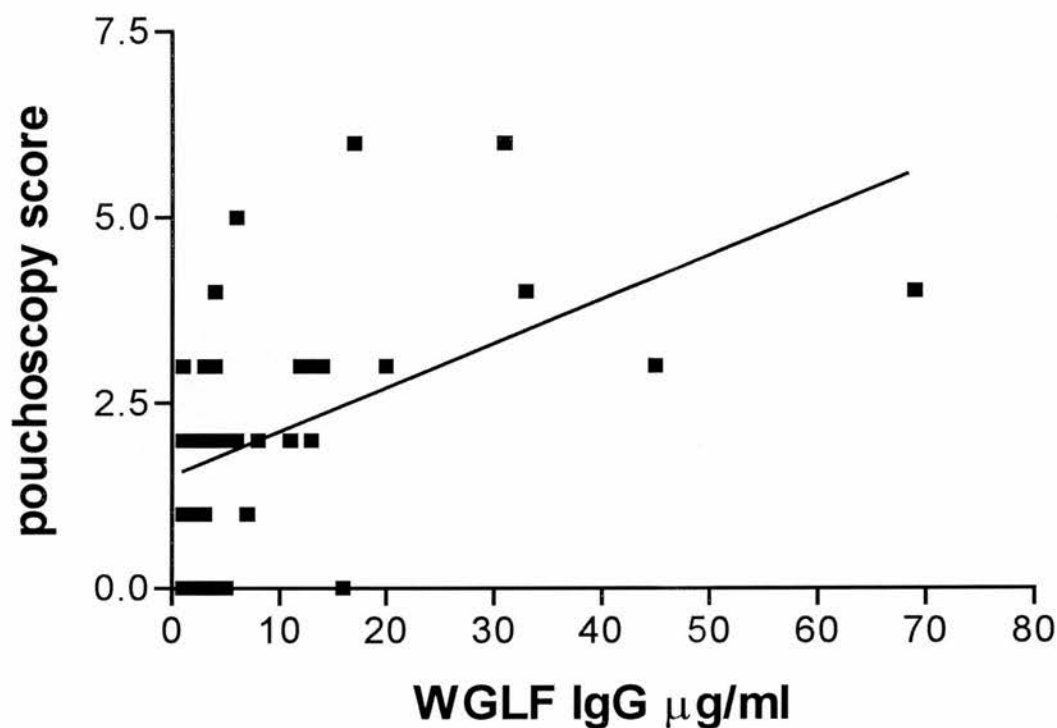
The values of IgG ranged from 1 to 69  $\mu\text{g/ml}$  (Figure 6.1). The WGLF IgG was higher in the 'early' group (patients who had their pouch functioning for less than six months) as compared to the rest of the UC patients ( $p=0.086$ ).

###### **6.3.A.1.2. WGLF IgG, Pouchoscopy & Pathology Findings**

###### **Pouchoscopy Score**

WGLF IgG correlated with the pouchoscopy score ( $r=0.50$ ,  $p=0.0001$ ; see Figure 6.2). The pouchoscopy score may vary from 0-6. If we separate the patients with a score 0-1 from the rest, these patients should have a 'normal' pouch endoscopically. The cut off point for WGLF IgG of  $10\mu\text{g/ml}$  is used by the GI laboratory to identify 'activity' in IBD. In the ileoanal pouches the WGLF





**Figure 6.2.** The pouchoscopy score related to the WGLF IgG (n=56,  $r=0.50$ ,  $p=0.0001$ )

**Table 6.2.** The pouchoscopy score & WGLF IgG in pouch patients. Patients with high WGLF IgG have high pouchoscopy score.

Pouchoscopy Score	0-1	2-6
WGLF IgG<10μg/ml	n=20	n=24
WGLF IgG≥10μg/ml	n=1*	n=12

\*The patient who had a WGLF IgG >10μg/ml, but low pouchoscopy score, is diabetic on NSAID, who had a poor functional score with high systemic WCC, platelet count, ESR and iron deficiency anaemia; the pouch biopsy characteristics were unremarkable.



IgG was invariably less than 10µg/ml in the subgroup with a pouchoscopy score 0-1 (see Table 6.2). For a higher pouch score ( $\geq 2$ ) the WGLF IgG varies.

### **Pouch Biopsy Neutrophilia**

WGLF IgG was higher in pouches with moderate/severe neutrophilia as compared to the pouches with minimal or with mild neutrophilia ( $p=0.0130$ ,  $p=0.0118$  respectively; see Figure 6.3). The patients with moderate/severe pouch neutrophilia and WGLF IgG < 10µg/ml: 3 out of 5 had a pouch score 0-1.

### **Chronic Inflammation in Pouch Biopsy**

WGLF IgG was lower in the subgroup with minimal changes in their biopsies. ( $P < 0.05$ , Figure 6.4). Patients with moderate/severe chronic inflammation but with WGLF IgG < 10µg/ml were all but one on metronidazole as compared to the ones with WGLF IgG  $\geq 10$ µg/ml ( $p=0.048$ ; see Table 6.3).

### **Pouch Biopsy Ulceration & Villous Atrophy**

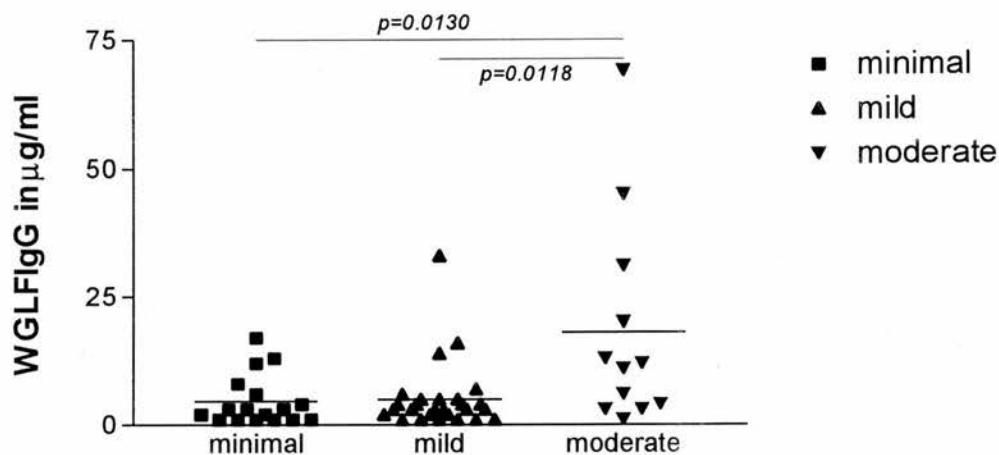
There was no apparent correlation between WGLF IgG and the presence of biopsy ulceration or villous atrophy.

## **6.3.A.1.3. WGLF IgG & PDAI**

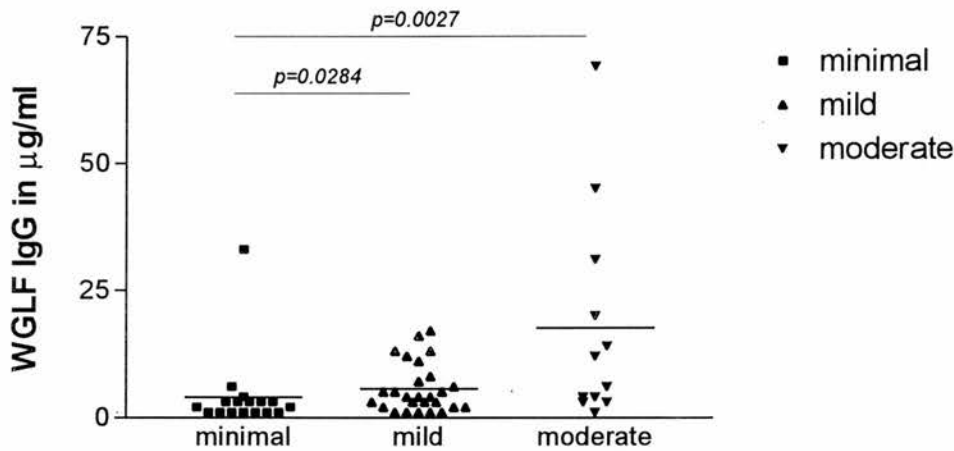
Since the pouchoscopy score and the biopsy characteristics are a part of the Pouchitis Disease Activity Index (PDAI), one may anticipate that there should be a relation between PDAI and WGLF IgG. There was a significant positive correlation between PDAI and WGLF IgG ( $r=0.58$ ,  $p < 0.0001$ , Figure 6.5).

Not all the patients with high PDAI had high WGLF IgG. Four patients had high PDAI but low IgG (see Table 6.4). Equally there were patients with high WGLF IgG but low PDAI score.

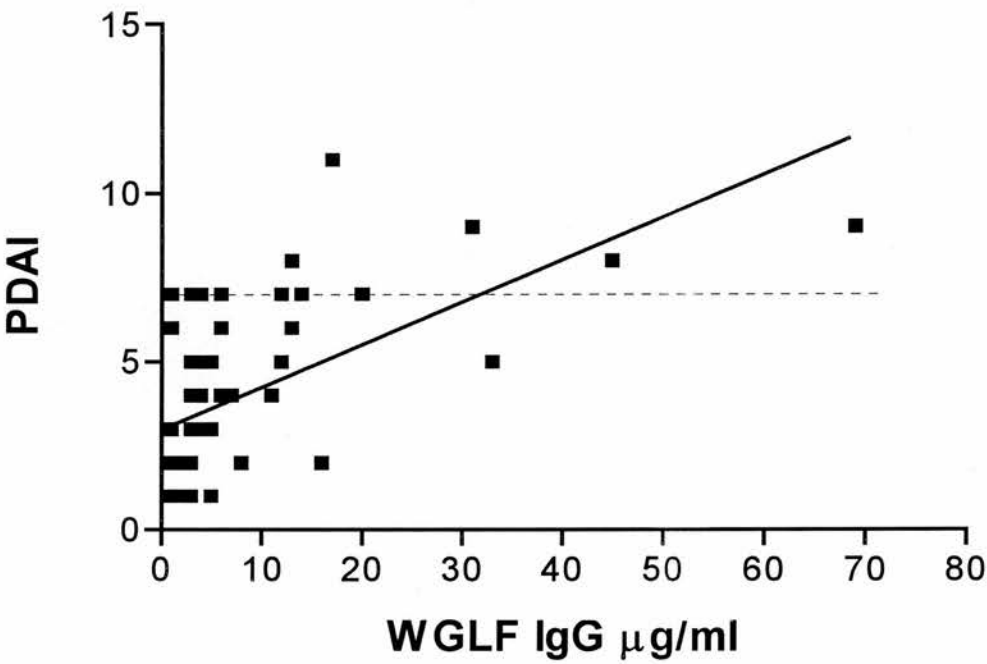
To summarise, **WGLF IgG** correlates with the disease activity index PDAI and also the pouchoscopy score and the pouch biopsy characteristics of neutrophilia and chronic inflammation.



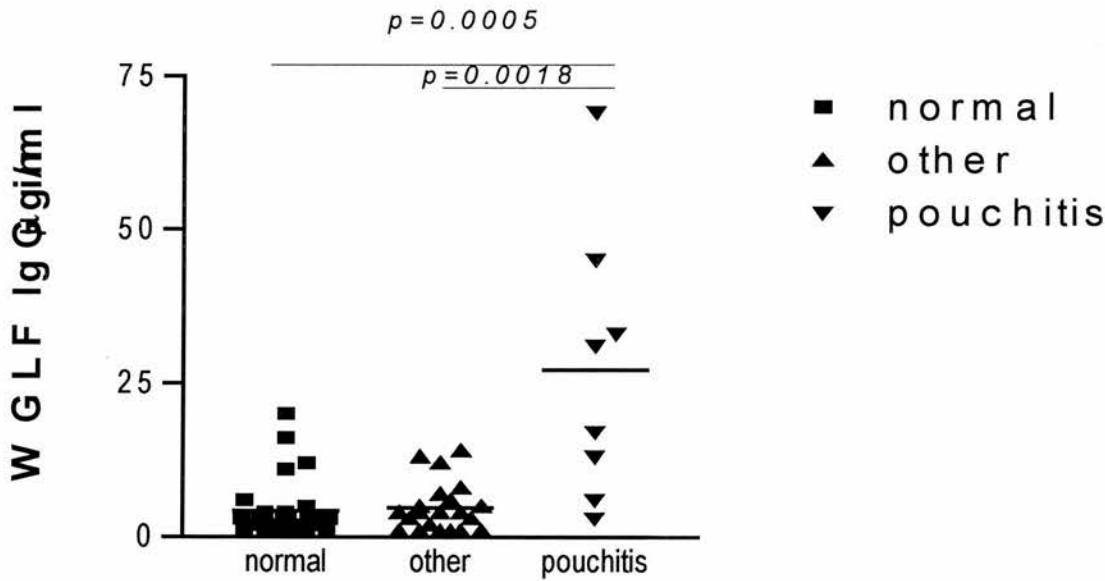
**Figure 6.3.** The biopsy neutrophilia and the WGLF IgG. The WGLF IgG was higher in patients with moderate biopsy neutrophilia as compared to those with minimal or mild neutrophilia in pouch biopsy.



**Figure 6.4.** The presence of chronic inflammation in pouch biopsies and the WGLF IgG. The WGLF IgG was higher in moderate and mild chronic inflammation as compared with patients with minimal chronic inflammation in pouch biopsy.



**Figure 6.5.** The WGLF IgG related to the PDAI score (n=56,  $r=0.58$ ,  $p<0.0001$ ). The dotted line represents PDAI of 7. Values of PDAI  $\geq 7$  define pouchitis.



**Figure 6.6.** WGLF IgG in the various groups: patients with pouchitis, with ‘other’ problems and normal pouches. Patients with pouchitis had higher WGLF IgG as compared with patients with ‘other’ problems or ‘normal’ pouches. Lines represent the mean of the samples.

**Table 6.3.** UC patients with moderate chronic Inflammation in pouches, the WGLF IgG and metronidazole treatment. The dose of metronidazole was higher in the subgroup with low WGLF IgG.

UC pouch patients with moderate chronic inflammation	WGLF IgG≥10µg/ml (n=6)	WGLF IgG<10µg/ml (n=6)	p value
Metronidazole mg/day	0 (0-400)	300 (0-600)	0.048

The results are presented as median (range). The Mann-Whitney test was used for comparison.

**Table 6.4.** The PDAI & WGLF IgG. Patients with low WGLF IgG had low PDAI.

	PDAI<7	PDAI≥7
WGLF IgG<10µg/ml	n=39	n=4*
WGLF IgG≥10µg/ml	n=5**	n=8

\*Patients with high PDAI but low WGLF IgG:

1. Patient on antipsychotic drugs
2. Patient complained of pouch dysfunction - a month later developed pouch vaginal fistula
3. Patient had pouchitis 2 months prior to study; clinically was improving still on metronidazole
4. Patient had an inflamed anal cuff with extension of the inflammation cephalad

\*\*Patients with low PDAI but high WGLF IgG:

- 1&2. Two patients with florid anal cuff inflammation, one had pyoderma gangrenosum as well
3. Patient had pouch dysfunction having had previous ileal resection for small bowel infarction; it was difficult for the patient to distinguish further deterioration of function,. Pouchoscopy and biopsy did not further increase the PDAI.
4. Patient was on 400 mg metronidazole, had pouch dysfunction, but not particularly disturbed pouchoscopy or biopsy results
5. Patient , diabetic on NSAID, had a high WCC, platelet count, ESR and iron deficiency anaemia but did not complain of any symptoms and neither had florid inflammation on pouchoscopy or biopsy (same patient as \* in table 6.2).

#### **6.3.A.1.4. WGLF IgG & Clinical Diagnosis**

During each session, after the pouchoscopy a decision was taken of whether this particular patient had 'pouchitis' or not. Other endoscopic and clinical entities were also considered, and a 'clinical diagnosis' was reached which did not actually had the benefit of histological confirmation (Figure 6.6). The PDAI was in good agreement with this global assessment, though two patients were ascribed to the 'other' group, later to have a PDAI calculated at 7. These were the patients \*1 & \*2 in Table 6.4.

#### **6.3.A.1.5. WGLF IgG: Stratification Of Pouch Patients**

##### **6.3.A.1.5.i The Three Subgroups**

Since the WGLF IgG may reflect disease activity in pouches, we used the IgG results to separate the patients of the UC group in 3 subgroups. In the Gastrointestinal laboratory WGLF IgG shows activity in IBD patients if  $\geq 10\mu\text{g/ml}$ , while in normal volunteers, outside hospital controls, a value of  $4\mu\text{g/ml}$  is commonly found.

There were patients who had very low WGLF IgG ( $\text{IgG} \leq 4\mu\text{g/ml}$ ;  $n=35$ ), who also had low values for all the other tests of WGLF. A medium WGLF IgG group, possibly 'abnormal' ( $\text{IgG}$ :  $5-9\mu\text{g/ml}$ ;  $n=8$ ) and a variety of range for the other results. Lastly, a third group had high IgG ( $\geq 10\mu\text{g/ml}$ ;  $n=13$ ).

##### **6.3.A.1.5.ii Comparisons Between Low & High WGLF IgG Subgroups**

The clinical characteristics that were different between the very low IgG ( $< 4\mu\text{g/ml}$ ,  $n=35$ ) and high IgG ( $\geq 10\mu\text{g/ml}$ ,  $n=13$ ) groups are given in Table 6.5a. In addition to that, the biopsy neutrophilia was worse in the high group ( $\chi^2=9.965$ ;  $p=0.007$ ). The chronic inflammation at pouch biopsy was greater in the high group ( $\chi^2=7.741$ ;  $p=0.021$ ). Lastly the high group had more anal cuff inflammation ( $\chi^2=4.255$ ;  $p=0.039$ ). See Table 6.5b.

**Table 6.5a.** The clinical parameters in the low ( $\text{IgG} \leq 4 \mu\text{g/ml}$ ) and high IgG ( $\text{IgG} \geq 10 \mu\text{g/ml}$ ) pouch groups. These were higher in the high WGLF IgG group.

Clinical parameters	Low IgG group ( $\text{IgG} \leq 4 \mu\text{g/ml}$ ) n=35	High IgG group ( $\text{IgG} \geq 10 \mu\text{g/ml}$ ) n=13	p value
PDAl score	2 (1-7)	6 (2-11)	0.0001
pouchoscopy score	2 (0-4)	3 (0-6)	0.0004
functional score	4 (0-11)	8 (1-14)	0.0095
bowel activity nocturnal	1 (0-3)	1.5 (0-7)	0.0463
WCC	5.8 (3.6-11.6)	9.5 (6.4-13.3)	0.0002
Platelet count	243 (153-454)	342 (137-588)	0.0051

**Table 6.5b.** The WGLF parameters in the low and high IgG pouch groups. These were higher in the high WGLF IgG group.

WGLF parameters	Low IgG group ( $\text{IgG} \leq 4 \mu\text{g/ml}$ ) n=35	High IgG group ( $\text{IgG} \geq 10 \mu\text{g/ml}$ ) n=13	p value
Albumin $\mu\text{g/ml}$	7 (1-22)	28 (12-69)	0.0001
AlAT $\mu\text{g/ml}$	4 (1-10)	8 (4-44)	0.0002
Hb $\mu\text{g/ml}$	2 (0.4-12)	7 (2-156)	0.0003
IL-1 $\beta$ pg/ml	4 (0-34)	59.8 (8-137)	0.0001
IL-8 pg/ml	43 (0-184)	548 (47-1000)	0.0001
IgA $\mu\text{g/ml}$	43 (1-105)	77 (31-318)	0.0028
IgM $\mu\text{g/ml}$	8 (1-76)	21 (2-154)	0.0050
GE $\mu\text{kat/l}$	<0.039 (0.039-0.079)	0.095 (0.039-0.511)	0.0008

The results are expressed as median (range). The Mann-Whitney test was used for comparison.

**Table 6.5c.** The clinical & WGLF parameters in the low and medium IgG pouch groups. The functional score and WGLF albumin & IL-8 were higher in the medium WGLF IgG group.

<b>WGLF / clinical parameters</b>	<b>Low IgG group (IgG≤4mg/ml) n=35</b>	<b>Medium IgG group (4&lt;IgG&lt;10mg/ml) n=8</b>	<b>p value</b>
WGLF albumin µg/ml	7 (1-22)	15 (3-25)	0.0178
WGLF IL-8 pg/ml	37 (0-184)	154 (55-907)	0.0006
functional score	4 (0-11)	7.5 (1-10)	0.0459

**Table 6.5d.** The clinical & WGLF parameters in the medium and high IgG pouch groups. These were higher in the high WGLF IgG group.

<b>WGLF / clinical parameters</b>	<b>Medium IgG group (4&lt;IgG&lt;10mg/ml) n=8</b>	<b>High IgG group (IgG≥10mg/ml) n=13</b>	<b>p value</b>
WGLF albumin µg/ml	15 (3-25)	28 (12-69)	0.0296
WGLF A1AT µg/ml	4 (1-6)	8 (4-44)	0.0017
WGLF IL-1β pg/ml	8.3 (0.7-143.0)	59.9 (8.8-137.4)	0.0390
WGLF IL-8 pg/ml	154.2 (55-907)	611.0 (47-1000)	0.0317
WGLF GE µkat/ml	<0.039 (0.039-0.042)	0.095 (0.039-0.511)	0.0165
pouchoscopy score	2 (0-5)	3 (0-6)	0.0302
PDAI	4 (1-7)	6 (2-11)	0.0447

The results are expressed as median (range). The Mann-Whitney test was used for comparison.

### **6.3.A.1.5.iii Comparisons Between Low & Medium WGLF IgG Subgroups**

The WGLF albumin of the medium group was higher compared to the low group ( $p=0.0178$ ). Similarly, the WGLF IL-8 was higher ( $p=0.0005$ ). The medium IgG group of patients had a worse functional score at the time of lavage ( $p=0.0459$ ). See Table 6.5c.

### **6.3.A.1.5.iv Comparisons Between Medium & High WGLF IgG Subgroups**

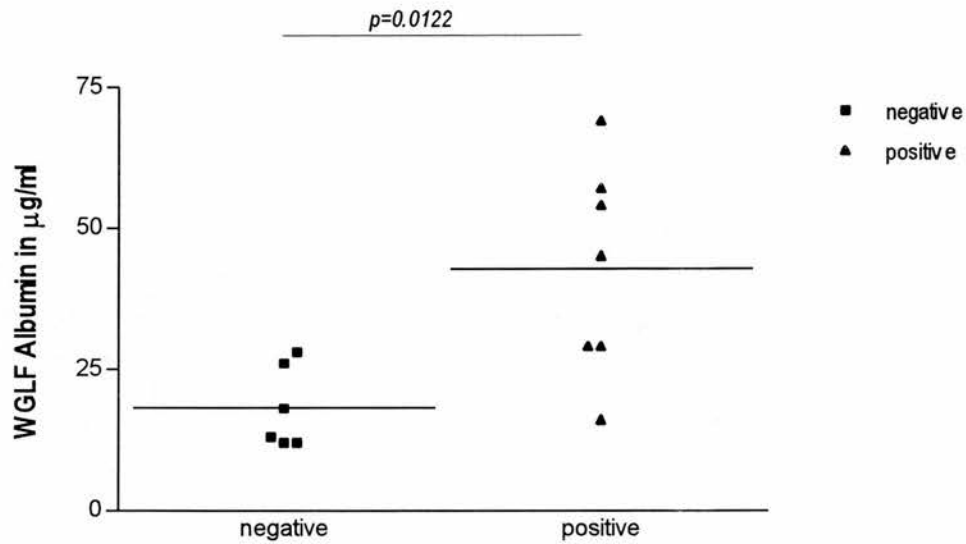
The WGLF albumin was lower in the medium IgG group as compared to the high group ( $p=0.0296$ ). The WGLF  $\alpha 1$ -antitrypsin was lower in the medium IgG group compared to the high group ( $p=0.0017$ ). The WGLF IL-8 in the medium group was lower compared to the high group ( $p=0.0277$ ). In addition, the medium group had lower IL- $1\beta$ , when compared to the high group ( $p=0.0464$ ). Granulocyte elastase was lower in the medium group, compared to the high group ( $p=0.0165$ ). The pouchoscopy score was worse for the high group ( $p=0.0302$ ) and also the PDAI score was higher ( $p=0.0447$ ) compared to the medium group. See Table 6.5d

### **6.3.A.1.5.v The High Group: Subdivisions According To Moskowitz Criteria**

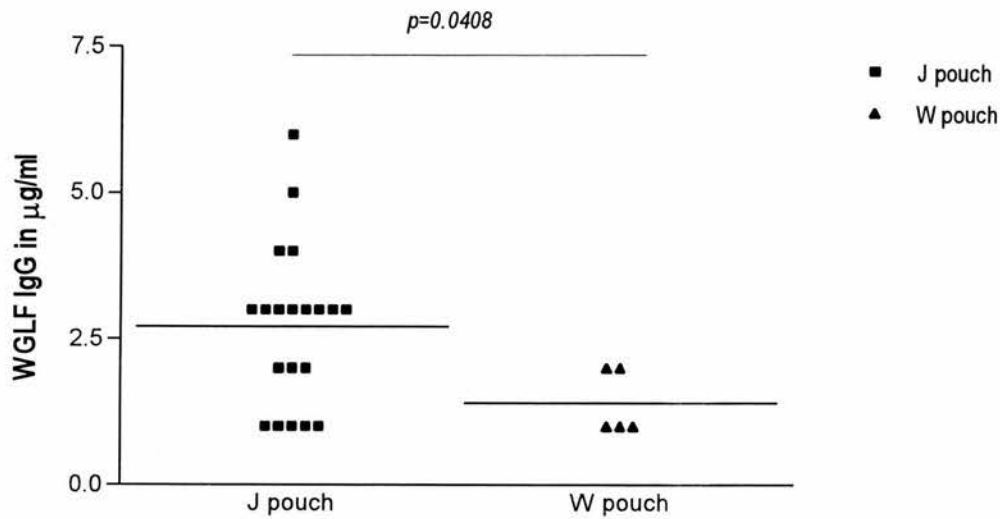
In the high WGLF IgG group ( $\text{IgG} \geq 10 \mu\text{g/ml}$ ), all patients with clinical active 'pouchitis' according to Moskowitz criteria were included ( $n=7$ ). When the 'positive' for pouchitis patients were compared with the rest of the high IgG ('negative' for pouchitis), the only difference in the WGLF parameters was albumin levels (Figure 6.7).

Other differences between the 'negative' and the 'positive' high IgG patients were mainly centred on the histological differences, on which the Moskowitz criteria heavily rely; the 'positive' group had worse biopsy neutrophilia ( $\chi^2=6.198$ ,  $p=0.013$ ), worse score for ulceration ( $\chi^2=4.952$ ,  $p=0.026$ ), and worse chronic inflammation ( $\chi^2=9.551$ ,  $p=0.002$ ). There was no significant difference in the amount of villous atrophy present in the pouch biopsy.





**Figure 6.7.** WGLF albumin in the high IgG group of patients with pouchitis ('positive') or without pouchitis ('negative') according to the Moskowitz Criteria. WGLF albumin was higher in patients with active pouchitis and high WGLF IgG.



**Figure 6.8.** The WGLF IgG in the 'J' pouch was higher as compared to the 'W' pouch.

The 'positive' group versus the 'negative' group was characterised by:

- a. less urgency ( $\chi^2= 8.4$ ,  $p=0.04$ )
- b. less evacuation difficulties ( $\chi^2= 5.182$ ,  $p=0.023$ );
- c. less history of bleeding per pouch ( $\chi^2= 4.286$ ,  $p=0.038$ ).
- d. more extraintestinal manifestations ( $\chi^2= 9.479$ ,  $p= 0.002$ ).
- e. more prone to change their diet more ( $\chi^2= 4.55$ ,  $p=0.033$ ).
- f. less narrow anastomoses on clinical examination ( $\chi^2= 6.741$ ,  $p=0.010$ ).

PDAI  $\geq 7$  was evident in all the 'positive' group patients, i.e. they had pouchitis according to the PDAI 'definition'.

#### **6.3.A.1.5.vi Comparisons Between 'High Group' & 'Low+Medium Group'**

To explore the relationship of the WGLF IgG and the other clinical characteristics of the patients, we combined the low and medium IgG groups (IgG  $<10\mu\text{g/ml}$ ; the 'combined' group) and compared them to the high IgG group (IgG  $\geq 10\mu\text{g/ml}$ ).  $10\mu\text{g/ml}$  is considered to be the laboratory's cut off point for IBD disease activity assessment.

Patients with high IgG ( $\geq 10\mu\text{g/ml}$ ) appeared to have more extraintestinal manifestations ( $\chi^2= 7.786$ ,  $p=0.005$ ; RR= 3.6; 95%CI: 1.46, 8.52). None of them had been treated immediately before the colectomy with high doses of steroids ( $\chi^2= 4.139$ ,  $p=0.042$ ; RR= 1.4; 95%CI: 1.01, 1.93). 'Atypia' described in the histopathology of their colectomy report was common ( $\chi^2= 4.15$ , with Yates correction,  $p=0.05$ ; RR= 3.5; 95%CI: 1.04, 11.68). Patients had to change their diet more often after pouch surgery ( $\chi^2= 10.055$ ,  $p=0.002$ ; RR= 5.1; 95%CI: 1.86, 13.96) and had worse social handicap ( $\chi^2= 4.998$ ,  $p=0.026$ ; RR= 2.8; 95%CI: 1.13, 6.9). Anal cuff inflammation was seen more often ( $\chi^2= 5.299$ ,  $p=0.022$ ; RR= 2.9; 95%CI: 1.17, 7.17).

#### **6.3.A.1.5.vii WGLF IgG & Comparisons Of Various Clinical Subgroups**

Patients with a history of extraintestinal manifestations had higher WGLF IgG ( $p=0.0113$ ). Patients with anal cuff inflammation had higher WGLF IgG ( $p=0.0032$ ). Patients who were complaining of day soiling and had social

**Table 6.6.** WGLF IgG in the subgroups of patients with present or absent clinical parameters. WGLF IgG was higher in patients with extraintestinal manifestations, anal cuff inflammation, day incontinence and social handicap.

Clinical parameters	WGLF IgG in patients with present parameters	WGLF IgG in patients with absent parameters	p value
EIM	6 (1-69) n=17	3 (1-33) n=39	0.0113
Anal cuff inflammation	6 (2-33) n=16	3 (1-69) n=40	0.0031
Day incontinence	4.5 (2-17) n=12	3 (1-69) n=44	0.0424
Social handicap	6 (1-45) n=13	3 (1-69) n=43	0.0180

The values are expressed as median (range)

Mann-Whitney test was used for comparison

handicap also had higher IgG ( $p=0.0424$ , &  $p=0.018$ , respectively). See Table 6.6.

#### **6.3.A.1.5.viii WGLF IgG & 'J' Or 'W' Pouch Configuration**

No patient with a 'W' pouch had clinical pouchitis, or had a value of WGLF IgG  $\geq 10\mu\text{g/ml}$ . In the 'combined' IgG group, patients with 'J' pouches appeared to have higher WGLF IgG than patients with 'W' ( $p=0.0137$ ). This difference was sustained even in the subgroup of clinically normal pouches ( $p=0.0408$ ). See Figure 6.8.

#### **6.3.A.1.5.ix. WGLF IgG & Previous Tonsillectomy**

The incidence of tonsillectomies recorded in the high IgG group was compared to that in the 'combined' IgG group. The group of high IgG had significantly more tonsillectomies in their past medical history ( $\chi^2=13.626$ ,  $p=0.001$ ; RR= 3.5; 95%CI: 1.56, 7.84).

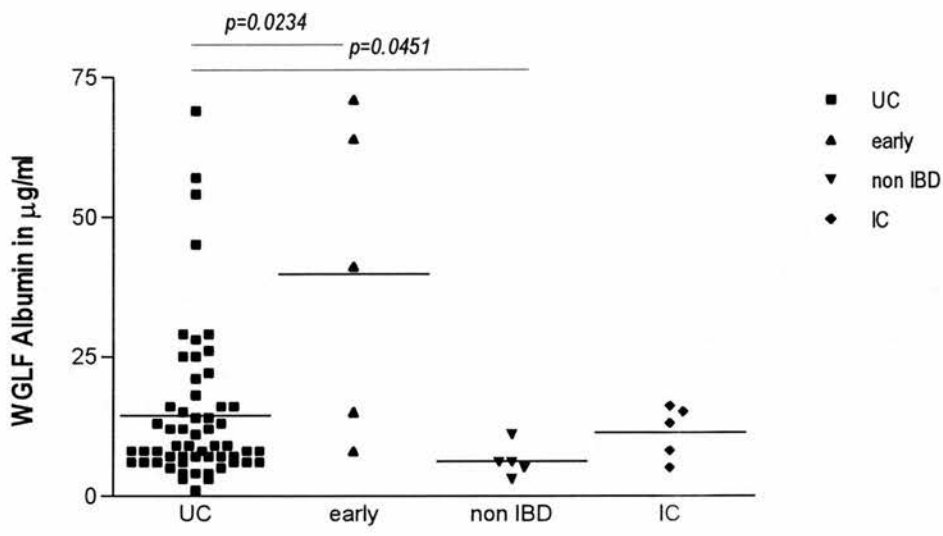
### **6.3.A.2. WGLF Albumin**

#### **6.3.A.2.1. WGLF Albumin In The Various Diagnostic Groups**

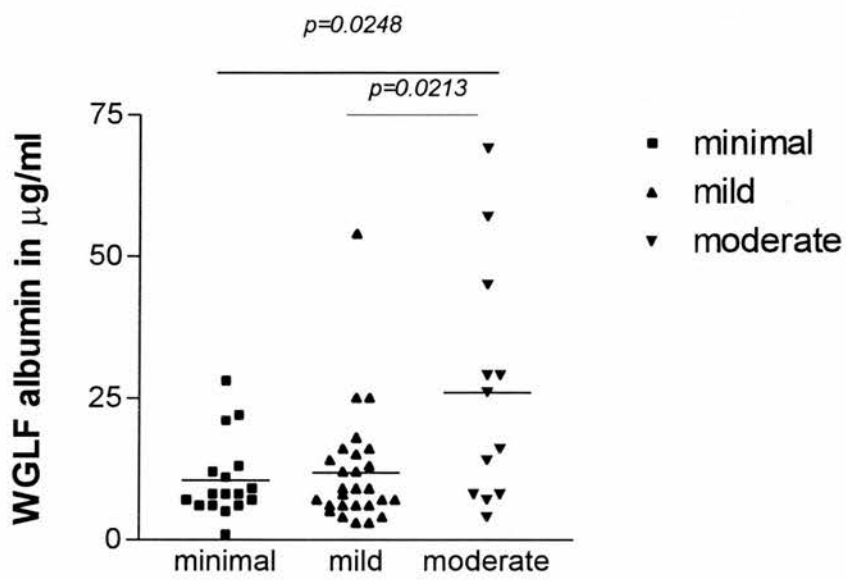
The WGLF albumin in the ileoanal pouches ranged from  $1\mu\text{g/ml}$  to  $71\mu\text{g/ml}$  (median:  $9\mu\text{g/ml}$ ; 95%CI 7.2, 12.8). The 'early' group had higher albumin compared to the main group of UC pouch patients ( $p=0.0234$ ). The 'non IBD' cases, however, had lower albumin ( $p=0.0451$ ). See Figure 6.9.

#### **6.3.A.2.2. WGLF Albumin & Inflammatory Parameters**

WGLF albumin correlated with blood WCC ( $r=0.35$ ,  $p=0.0193$ ), and the pouchoscopy score ( $r=0.47$ ,  $p=0.0002$ ).



**Figure 6.9.** The WGLF albumin in the various diagnostic groups. WGLF albumin was higher in the ‘early’ group as compared to the UC group. The albumin in the UC group was higher as compared to the ‘non IBD’ group.



**Figure 6.10.** WGLF albumin in patients according to biopsy neutrophilia. Albumin was higher in the subgroup with moderate neutrophilia as compared to mild or minimal neutrophilia.

The WGLF albumin was higher in patients with moderate/severe neutrophilia in pouch biopsy compared to those with mild or minimal neutrophilia ( $p=0.0213$ , and  $p=0.0248$ , respectively; see Figure 6.10).

Furthermore, the WGLF albumin was higher in patients with moderate chronic inflammatory changes in the pouch biopsy ( $p=0.0404$ ; see Figure 6.11).

#### **6.3.A.2.3. WGLF Albumin, PDAI & The Clinical Diagnosis**

The WGLF albumin correlated with the PDAI ( $r=0.52$ ,  $p<0.0001$ ; Figure 6.12) and also with the investigator's overall assessment of the pouch inflammation (Figure 6.13).

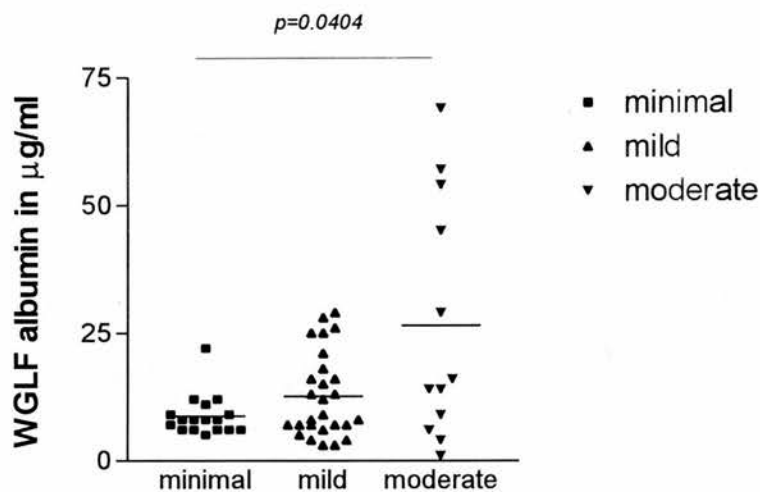
#### **6.3.A.2.4. WGLF Albumin & The Various WGLF IgG Groups**

The levels of WGLF albumin are presented in the three subgroups ('low'  $\text{IgG} \leq 4 \mu\text{g/ml}$ ; 'medium'  $\text{IgG}: 5-9 \mu\text{g/ml}$ ; 'high'  $\text{IgG} \geq 10 \mu\text{g/ml}$ ; see section 6.3.A.1.5.ii) in Table 6.7. The albumin levels followed very closely the general pattern of low, medium and high protein loss as described by the WGLF IgG. Furthermore, the WGLF albumin in the 'high' WGLF IgG subgroup was the main parameter distinguishing the 'positive' pouchitis from the 'negative' according to the Moskowitz criteria ( $p=0.0122$ ; see section 6.3.A.1.5.iii).

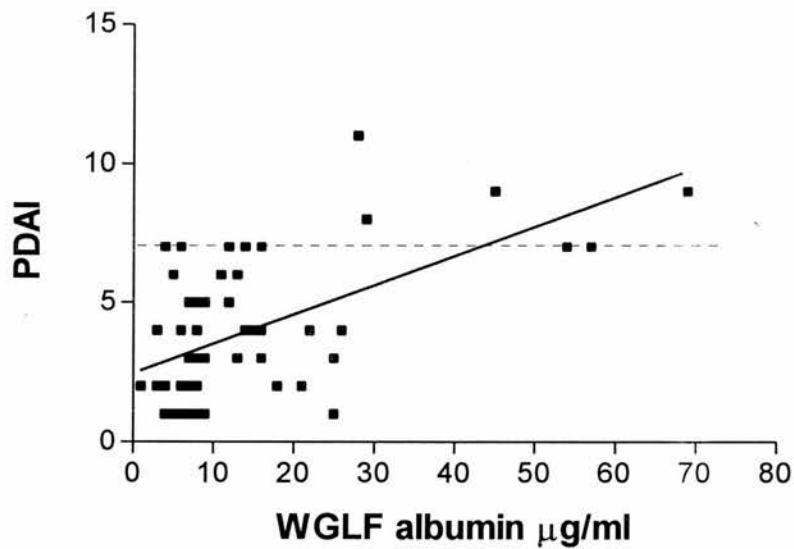
#### **6.3.A.2.5. WGLF Albumin: Stratification Of Pouch Patients**

The cut off point for WGLF albumin is  $26 \mu\text{g/ml}$ . Eight patients had values above this level. These all had high WGLF IgG. Patients, however, with relatively increased levels of WGLF albumin (but less than  $26 \mu\text{g/ml}$ ) were present in both the other two groups of medium and low IgG.

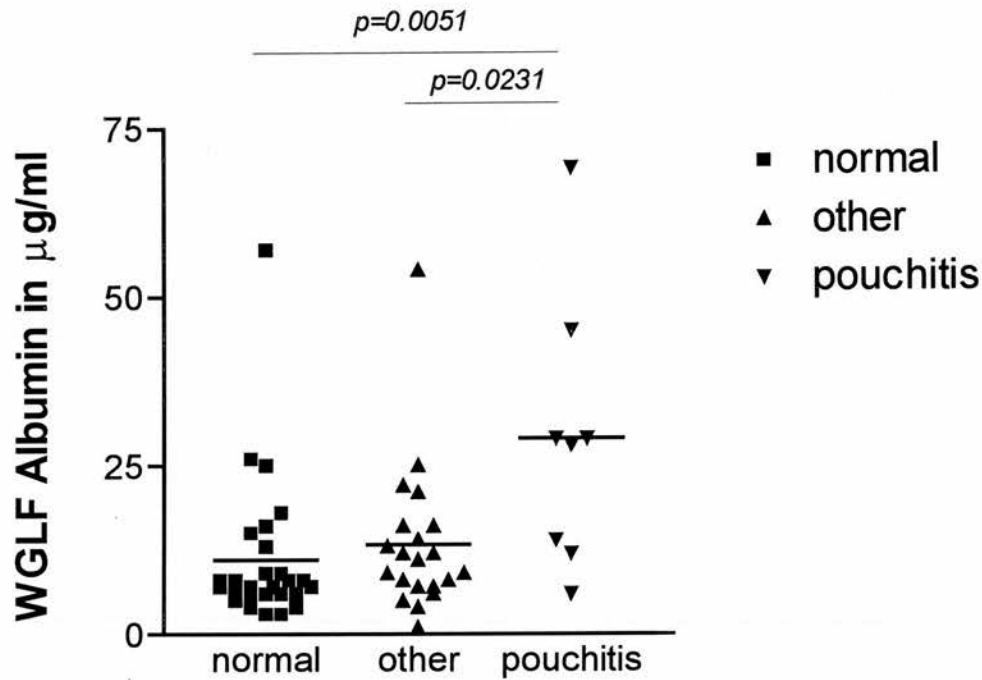
In the previous section 6.3.A.1.5.iv the medium and low IgG groups were combined, in order to elicit some differences. The same principle was followed here. In order to discover the characteristics of these patients with the relatively high WGLF albumin, patients were divided first according to the WGLF IgG ( $\leq 10$  or  $\geq 10 \mu\text{g/ml}$ ) and then according to albumin level ( $\leq 10$  or  $\geq 10 \mu\text{g/ml}$ ).



**Figure 6.11.** WGLF albumin results according to the degree of chronic inflammation in the biopsy. Albumin was higher in patients with moderate chronic inflammation as compared to those with minimal chronic inflammation.



**Figure 6.12.** WGLF albumin relates to the PDAI ( $n=56$ ,  $r=0.52$ ,  $p<0.0001$ ). The dotted line represents PDAI of 7. Values of PDAI  $\geq 7$  signify pouchitis.



**Figure 6.13.** WGLF albumin in the various groups according to clinician’s overall assessment of pouch inflammation. Albumin was higher in the pouchitis group as compared with the ‘other’ group or patients with normal pouch.

**Table 6.7** The WGLF albumin in the low, medium and high WGLF IgG groups. Albumin was higher in the high as compared to the medium or the low IgG groups.

WGLF Parameter	Low IgG group (IgG $\leq$ 4 $\mu\text{g/ml}$ ) n=35	Medium IgG (IgG: 5-9 $\mu\text{g/ml}$ ) n=8	High IgG group (IgG $\geq$ 10 $\mu\text{g/ml}$ ) n=13	p value
Albumin $\mu\text{g/ml}$	7 (1-22)	15 (3-25)	28 (12-69)	0.0178* 0.0296** 0.000***

The results are expressed as median (range). Mann-Whitney test was used for comparison

- \* low versus medium WGLF IgG group
- \*\* medium versus high WGLF IgG group
- \*\*\* low versus high WGLF IgG group



**Table 6.8a.** Clinical parameters in UC pouch patients with WGLF IgG<10µg/ml and high (LH) or low (LL) WGLF albumin groups. Cut off point for albumin: 10µg/ml. The PDAI, functional & pouchoscopy scores were higher in the high albumin group.

<b>Parameter</b>	<b>LH group</b> (n=12)	<b>LL group</b> (n=31)	<b>p value</b>
Pouchoscopy score	2 (0-5)	1 (0-4)	0.0443
PDAI	4 (1-7)	2 (1-7)	0.0453
Functional score	7 (1-10)	4 (0-11)	0.0480

**Table 6.8b** The PDAI in UC pouch patients with WGLF IgG≥10µg/ml & high (HH) or low (HL) WGLF albumin groups. Cut off point for albumin: 26µg/ml. The PDAI was higher in the high albumin group.

<b>Parameter</b>	<b>HH group</b> (n=8)	<b>HL group</b> (n=5)	<b>p value</b>
PDAI	8 (4-11)	5 (2-7)	0.0321

**Table 6.9.** WGLF albumin was higher in patients with present clinical parameters as compared to those with absent clinical parameters.

<b>Clinical parameters</b>	<b>WGLF albumin</b> in patients with present parameters	<b>WGLF albumin</b> in patients with absent parameters	<b>p value</b>
Day incontinence	14.5 (6-29) n=12	8 (1-69) n=44	0.0383
Stenosis pouch anal anastomosis	6.5 (3-12) n=8	9 (1-69) n=48	0.0308
Routine operation	12.5 (3-69) n=32	7 (1-45) n=24	0.0128

Using the principle of the Latin Square, the patients in the UC group were divided according to their albumin and IgG in the WGLF in four groups:

1. The first group (**LL**) had low WGLF IgG and albumin (n=31)
2. The second (**LH**) had relatively low IgG, i.e. below 10 $\mu$ g/ml, but relatively high albumin  $\geq$  10 $\mu$ g/ml (n=12)
3. The third group (**HL**) was characterised by high (>10 $\mu$ g/ml) IgG, but relatively low albumin <26 $\mu$ g/ml (n=5)
4. The fourth group had both IgG and albumin ( $\geq$ 26 $\mu$ g/ml) high (**HH**; n=8)

#### **The LL and the LH groups**

The biopsy characteristics were not significantly different, however the LH group had a higher pouchoscopy score (p=0.043). Similarly the PDAI score was higher (p=0.0453). The functional score was also higher (p=0.0480). The day soiling appeared to be the main functional characteristic different between the two groups ( $\chi^2=5.846$ , p=0.016). See Table 6.8a.

#### **The HL and HH groups**

The PDAI was significantly higher in the HH group (p=0.0321). The rest of the clinical, endoscopic, biopsy and WGLF parameters were not significantly different. See Table 6.8b.

### **6.3.A.2.6. WGLF Albumin & Other Observations**

There was a correlation observed between the albumin of WGLF and the use of loperamide, which was not seen between IgG and loperamide (r=0.32, p=0.0146). The WGLF albumin differed between patients who complained of day soiling or otherwise (p=0.0383). Patients who had a tight pouch anal anastomosis, had lower albumin in their WGLF (p=0.0308). Lastly, patients who underwent routine colectomies appeared to have higher WGLF albumin (p=0.0128). See Table 6.9.

### **6.3.A.3. WGLF $\alpha$ 1-Antitrypsin**

#### **6.3.A.3.1. WGLF $\alpha$ 1-Antitrypsin & Various Diagnostic Groups**

The  $\alpha$ 1- antitrypsin (A1AT), was considered as a marker of protein gut loss in the WGLF. The WGLF A1AT in the UC pouch patients ranged from 1 $\mu$ g/ml to 41 $\mu$ g/ml (median: 4.5 $\mu$ g/ml; 95%CI: 4.0, 6.0). Outwith results were: one case of pouchitis (WGLF IgG 45 $\mu$ g/ml, albumin 29 $\mu$ g/ml); and another with a relatively normal pouch and is referred previously in Tables 6.2 (\*) and 6.4 (\*\*) (WGLF IgG 16 $\mu$ g/ml, albumin 18 $\mu$ g/ml; diabetic patient on NSAIDs, with high WCC, platelets, ESR and iron deficiency anaemia). There were no statistical differences in the four diagnostic categories: UC pouch patients, 'early' UC pouch patients, IC pouch patients and 'non IBD' pouch patients (Figure 6.14).

#### **6.3.A.3.2. WGLF A1AT & The Various WGLF IgG Groups**

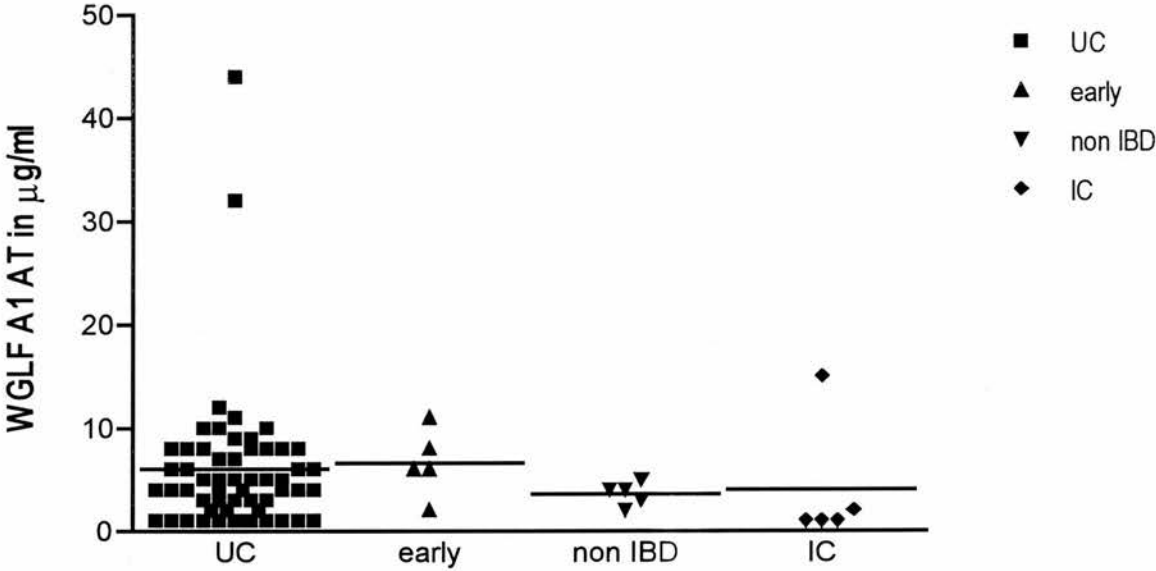
In the previous section 6.3.A.1.5.ii, the pouch patients were stratified according to the disease activity as measured by the WGLF IgG into three groups: low IgG ( $\leq$ 4 $\mu$ g/ml), medium IgG (5-9 $\mu$ g/ml), high ( $\geq$ 10 $\mu$ g/ml). The levels of A1AT in the three groups were also described.. The A1AT levels in the three groups are presented again in Table 6.10.

### **6.3.B. Bleeding In Ileoanal Pouches**

#### **6.3.B.1. WGLF Haemoglobin**

##### **6.3.B.1.1. WGLF Haemoglobin & The Various Diagnostic Groups**

Whole gut lavage was also used to assess the bleeding diathesis of pouch patients, by measuring haemoglobin (Hb) in the specimens provided. The WGLF



**Figure 6.14.** WGLF A1AT in the various diagnostic groups\*. There was no difference in the various groups.

\*‘UC group’ - patients who underwent restorative proctocolectomy for ulcerative colitis  
‘early UC group’ - patients who underwent restorative proctocolectomy for UC within the last 6 months  
‘IC group’ - patients who underwent restorative proctocolectomy for indeterminate colitis  
‘non IBD group’ - patients underwent restorative proctocolectomy for causes other than IBD

**Table 6.10.** The WGLF A1AT in the low, medium & high WGLF IgG groups. The A1AT was higher in the high WGLF IgG group as compared to the rest subgroups.

WGLF A1AT	Low IgG group IgG≤4µg/ml n=35	Medium IgG group IgG 5-9µg/ml n=8	High IgG group IgG≥10µg/ml n=13	p value
A1AT µg/ml	4 (1-10)	4 (1-6)	8 (4-44)	0.002** 0.0002***

\*\* medium versus high WGLF IgG group  
\*\*\* low versus high WGLF IgG group  
The results are expressed as median (range)  
Mann-Whitney test was used for comparison

Hb ranged from 0.4 µg/ml to 199 µg/ml. There were no significant differences between the miscellaneous diagnostic categories: UC pouch patients, 'early' group, IC patients and 'non IBD' group (Figure 6.15).

### **6.3.B.1.2. Haemoglobin, PDAI & Inflammatory Parameters**

There was no correlation with the systemic Hb. Neither did patients, who admitted that at times they had noticed blood in their motions, have higher WGLF Hb. The WGLF Hb did not relate with any other of the clinical parameters, parameters of inflammation or the PDAI.

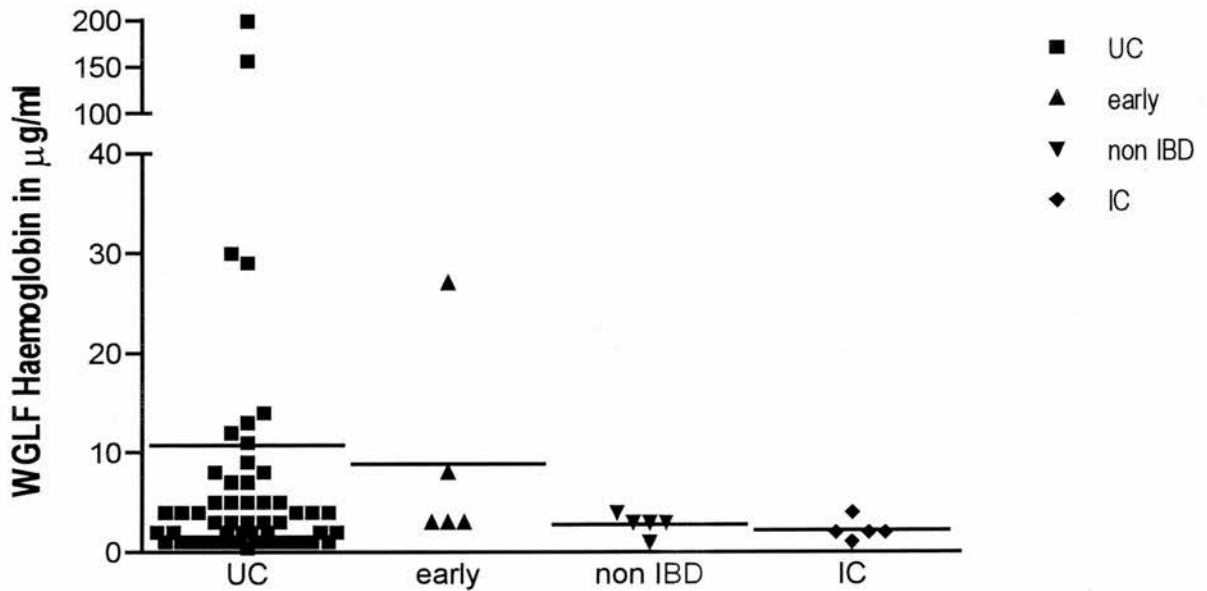
### **6.3.B.1.3. WGLF Haemoglobin: Stratification Of Pouch Patients**

#### **6.3.B.1.3.i High & Low WGLF Haemoglobin Groups**

Since bleeding may be a phenomenon not always related to gut protein loss, or disease activity, it was decided to separate the patients according to the WGLF Hb with a cut off point of 5 µg/ml. The value 5 µg/ml was selected because it is the reference value used in the laboratory for establishing significant bleeding at gut lavage, as mentioned above. With this separation indeed the two groups of low WGLF Hb (n=38) and high Hb (n=18) were significantly different with regard to the gut lavage parameters (Table 6.11).

### **6.3.B.1.4. Characteristics Of Patients With Very High WGLF Hb**

It was noticeable that five patients (4 in the UC group, and 1 in the 'early' group) had distinctly high WGLF Hb. Two of them had pouchitis, but not on metronidazole. The other two patients, were on metronidazole, one being only 6 weeks postoperatively, the other having pyoderma gangrenosum from florid anal cuff inflammation. The fifth patient was essentially 'normal' in other effects, but known to suffer from long-standing iron deficiency anaemia of unknown



**Figure 6.15.** WGLF Hb in the various diagnostic groups.

**Table 6.11.** The WGLF parameters in the groups with low and high WGLF Hb. These parameters were higher in the high WGLF Hb group as compared to the low WGLF Hb.

WGLF parameters	Low Hb n=38	High Hb n=18	p value
IgG $\mu\text{g/ml}$	3 (1-17)	9.5 (3-69)	0.0005
Albumin $\mu\text{g/ml}$	8 (1-57)	15.5 (3-69)	0.0217
A1AT $\mu\text{g/ml}$	4 (1-10)	8 (1-44)	0.0029
IL-8 pg/ml	53 (0-906)	294 (23-1000)	0.0111
IgA $\mu\text{g/ml}$	42 (1-153)	64 (40-318)	0.0003
IgM $\mu\text{g/ml}$	8 (1-76)	18 (8-154)	0.0011
GE $\mu\text{kat/l}$	<0.039 (0.039-0.226)	0.0230 (0.039-0.511)	0.0017

The results are expressed as median (range)

Mann-Whitney test was used for comparison

aetiology (Hb 103 g/l; WGLF IgG: 6µg/ml, albumin: 3µg/ml, A1AT: 6µg/ml, Hb: 199µg/ml).

The WGLF characteristics of the above 5 patients were very similar to the characteristics of the other high WGLF Hb patients. Most noticeably they presented with higher WGLF IgG,  $\alpha$ 1- antitrypsin, IL-1 $\beta$ , IL-8 and higher PDAI score compared to the rest of the patients. They did not seem to differ in their functional characteristics, or their systemic Hb.

### **6.3.C WGLF Cytokine Profile Of Ileoanal Pouches**

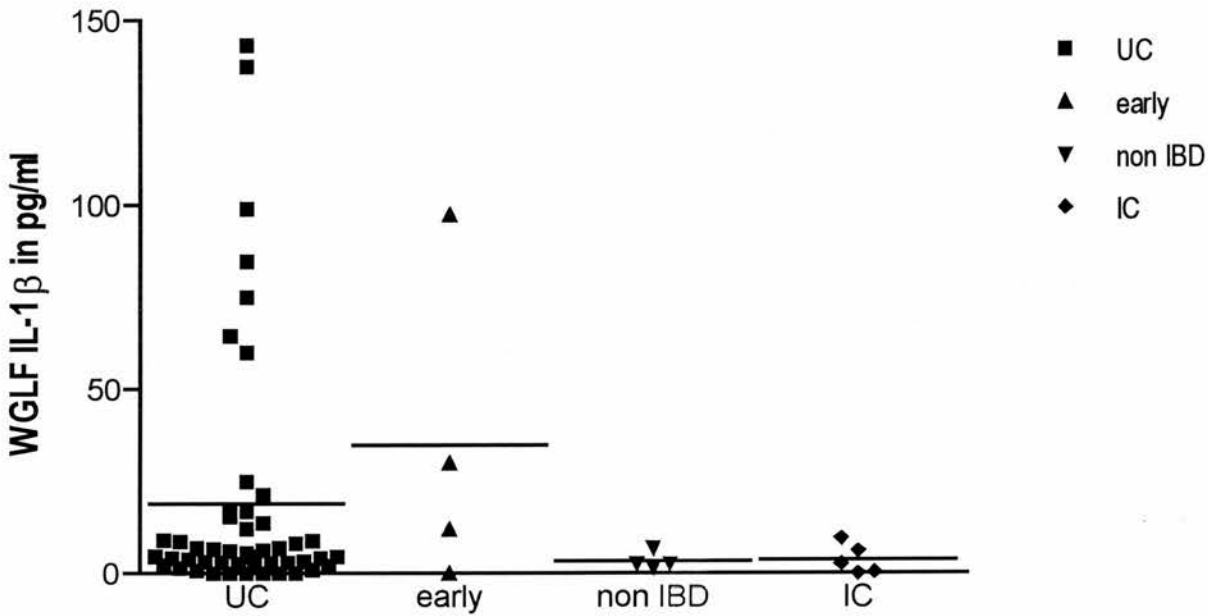
#### **6.3.C.1. The WGLF Interleukin IL-1 $\beta$**

##### **6.3.C.1.1. WGLF IL-1 $\beta$ In The Various Diagnostic Groups**

The interleukins IL-1 $\beta$  and IL-8, that are involved in many proinflammatory reactions, are readily measured in WGLF. The WGLF IL-1 $\beta$  in pouches ranged from 0 pg/ml to 143 pg/ml (median: 6.7 pg/ml; 95%CI: 4.26, 9.62) for the UC group, with no statistical differences between the diagnostic categories: UC pouch patients, 'early' group, IC patients and 'non IBD' group (Figure 6.16).

##### **6.3.C.1.2. WGLF IL-1 $\beta$ , PDAI & Inflammatory Parameters**

The WGLF IL-1 $\beta$  correlated with the disease activity index PDAI ( $r=0.37$ ,  $p=0.0037$ ), but also related to many other parameters of the clinical sphere. Most noticeable was the relationship with the blood WCC ( $r=0.61$ ,  $p<0.0001$ ); the platelet count ( $r=0.42$ ,  $p=0.0020$ ); and the ESR ( $r=0.42$ ,  $p=0.0032$ ). The pouchoscopy score correlated to the WGLF IL-1 $\beta$  ( $r=0.32$ ,  $p=0.0192$ ).



**Figure 6.16.** WGLF IL-1 $\beta$  in the various diagnostic groups.

**Table 6.12.** WGLF IL-1 $\beta$  in patients with present or absent clinical parameters. The WGLF IL-1 $\beta$  was higher in patients with diet change, social handicap and perianal discomfort.

Clinical parameters	WGLF IL-1 $\beta$ in patients with present parameter	WGLF IL-1 $\beta$ in patients with absent parameters	p value
Diet change	16.1 (0-137.4) n=34	4.6 (0-143.1) n=22	0.0470
Social handicap	12.1 (0-137.4) n=13	4.6 (0-143.1) n=43	0.0414
Perianal discomfort	8.9 (0-143.1) n=31	3.2 (0-120.0) n=25	0.0092

The results are expressed as median (range)

Mann-Whitney test was used for comparison



### 6.3.C.1.3. WGLF IL-1 $\beta$ & The Functional Parameters

The WGLF IL-1 $\beta$  related to the functional score at the time of the gut lavage and to related to night bowel activity ( $r=0.28$ ,  $p=0.0384$ ; and  $r=0.44$ ,  $p=0.0008$ , respectively).

Patients, who reported change in their diet after pouch surgery, had higher WGLF IL-1 $\beta$  ( $p=0.0047$ ). Similarly, patients with a considerable social handicap had also higher WGLF IL-1 $\beta$  ( $p=0.0228$ ). Likewise, patients who complained of perianal discomfort had again higher WGLF IL-1 $\beta$  ( $p=0.0035$ ). See Table 6.12.

### 6.3.C.1.4. WGLF IL-1 $\beta$ & The Clinical Diagnosis

The WGLF IL-1 $\beta$  levels were scattered independently of the clinical impression of pouchitis or not, as illustrated in Figure 6.17. In the 'pouchitis' group, the two patients who had low WGLF IL-1 $\beta$  had also low WGLF IgG, while the third one was already on maximum treatment with Ciproxin, Metronidazole and TPN one week prior to the WGLF. The WGLF IgG was raised, while the WGLF IL-8 was relatively elevated (200pg/ml).

The patient who was thought to be 'normal' and had high WGLF IL-1 $\beta$ , was anaemic (Hb: 10.4g/l), with raised systemic platelet count ( $535 \times 10^9/l$ ), slightly raised ESR (23mm/h), but essentially normal pouchoscopy and biopsy characteristics. This patient was diabetic and on Voltarol® for arthritis. The rest of the WGLF parameters were equally raised (WGLF IgG: 16 $\mu$ g/ml, albumin: 18 $\mu$ g/ml, A1AT: 44 $\mu$ g/ml, Hb: 11 $\mu$ g/ml, GE: 0.176 $\mu$ kat/l; see Tables 6.2 and 6.4).

The patient with the very high IL-1 $\beta$ , belonging in the 'other' group was a patient with a perianal abscess at the time of lavage. The rest of the WGLF parameters were: IgG: 8 $\mu$ g/ml, albumin: 21 $\mu$ g/ml, A1AT: 5 $\mu$ g/ml, Hb: 13 $\mu$ g/ml. The patient made initially a good recovery following drainage of the abscess, to develop later persistent perianal sepsis.



The three other patients were :

1. One patient with anal cuff inflammation and pyoderma gangrenosum (see Table 6.4)
2. One patient with previous ileal resection following pouch construction, with pouch dysfunction, who on the day of the lavage had high platelet count and ESR. The pouchoscopy and biopsy characteristics were not particularly abnormal, giving eventually a PDAI score of 5. The WGLF IgG was raised ( $12\mu\text{g/ml}$ ) as well as the WGLF Hb ( $7\mu\text{g/ml}$ ) and the neutrophil GE ( $0.095\mu\text{kat/l}$ ). See Table 6.4.
3. One patient with pouch dysfunction, and troublesome night frequency, and had 4 previous episodes of pouchitis. At the time of lavage the pouchoscopy and biopsy characteristics gave a PDAI score of 5. The WGLF parameters were not deranged (WGLF IgG:  $5\mu\text{g/ml}$ ). The patient was on 600mg of metronidazole long term.

### **6.3.C.2. The WGLF Interleukin IL-8**

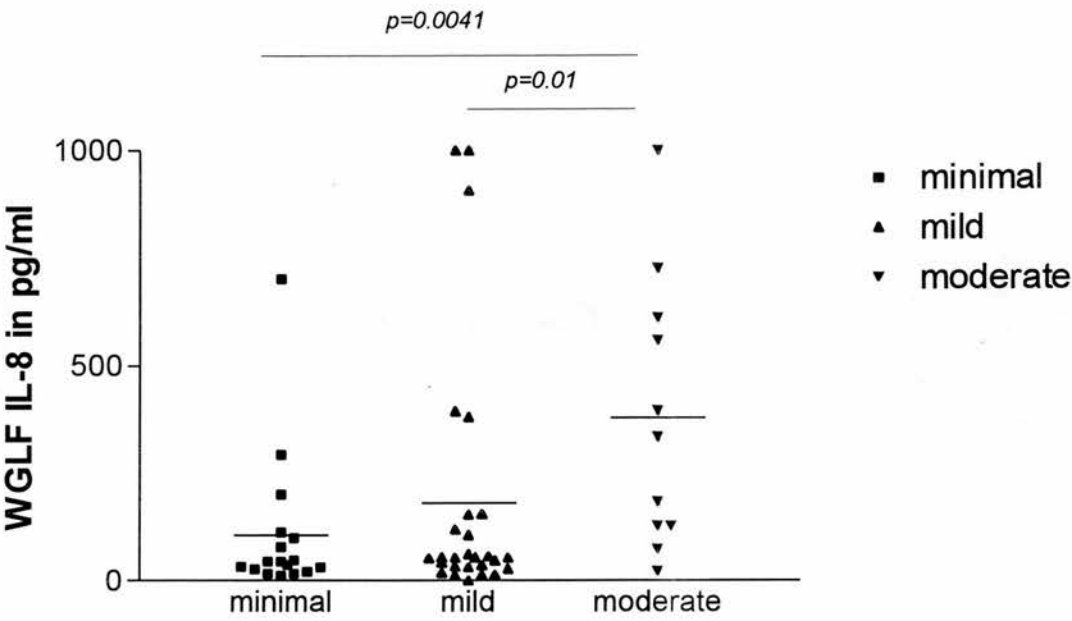
#### **6.3.C.2.1. WGLF IL-8 In The Various Diagnostic Groups**

The second cytokine measured in the WGLF for pouch patients was IL-8. The WGLF IL-8 ranged from 0 to 1000 pg/ml. The WGLF IL-8 in the 'early' group was not significantly higher from that of the UC group as a whole (Figure 6.18).

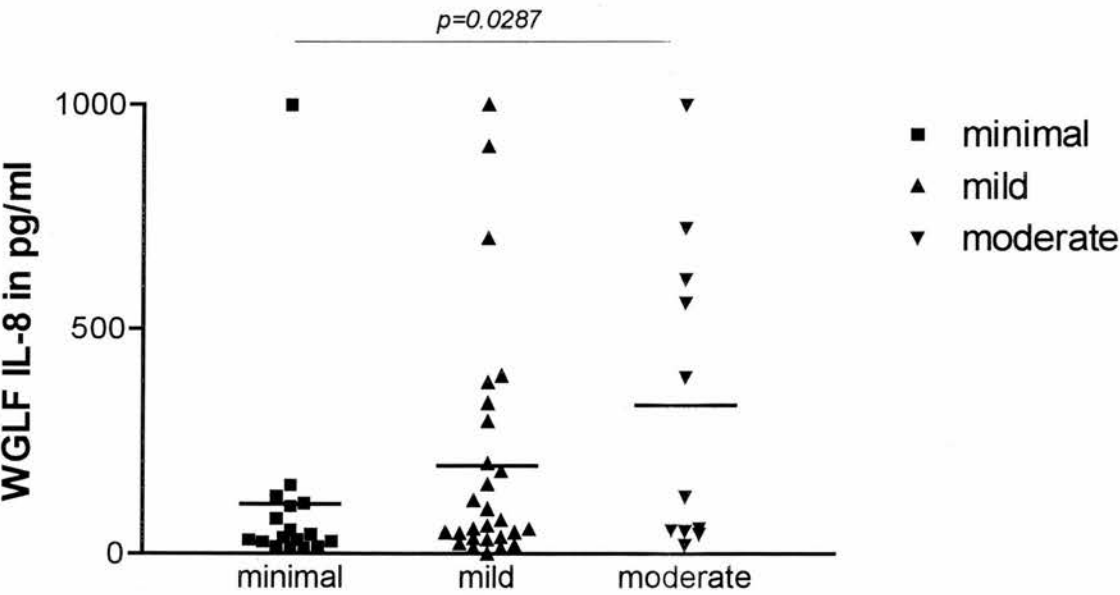
#### **6.3.C.2.2. WGLF IL-8, PDAI & Inflammatory Parameters**

The PDAI, as a measure of disease activity, correlated to the IL-8 ( $r=0.37$ ,  $p=0.0046$ ). On the other hand, haematological indices of inflammation were related to the IL-8: blood WCC ( $r=0.59$ ,  $p<0.0001$ ); and platelet count ( $r=0.51$ ,  $p=0.0003$ ). The pouchoscopy score correlated to the IL-8 ( $r=0.33$ ,  $p=0.0233$ ).

The biopsy neutrophilia was related to the WGLF IL-8. Patients with



**Figure 6.19.** WGLF IL-8 in pouch patients with variable biopsy neutrophilia. Patients with moderate neutrophilia had higher levels of WGLF IL-8..



**Figure 6.20.** WGLF IL-8 in patients with variable pouch chronic inflammation. The WGLF IL-8 was higher in patients with moderate chronic inflammation.

moderate neutrophil infiltration had higher WGLF IL-8. Figure 6.19 shows these differences between the patients with minimal (44.3 pg/ml), mild (53.7 pg/ml) and moderate/severe neutrophilia. The patients who had minimal or mild neutrophilia and high levels of WGLF IL-8 were those described previously in section 6.3.C.1.4.

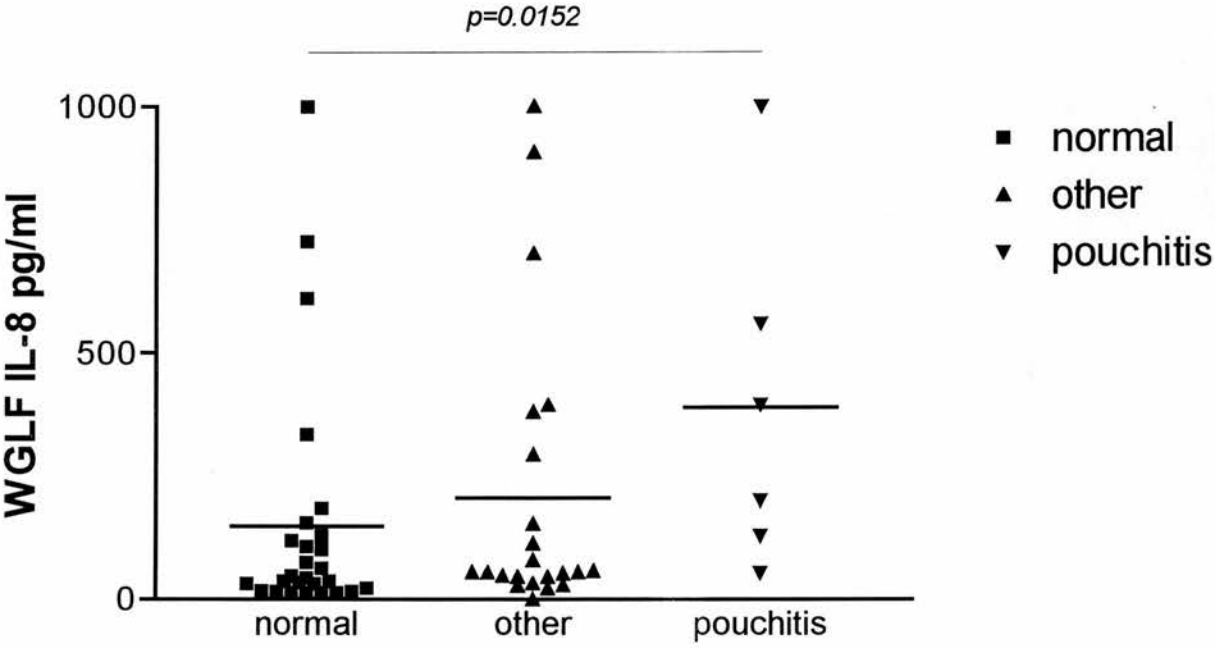
Similar differences were noted with the chronic inflammation in biopsies and the IL-8. Figure 6.20 presents these variances. The group of patients with moderate/severe chronic inflammation had higher IL-8 compared to those with minimal changes. The patients who had minimal or mild chronic inflammation but elevated WGLF IL-8 were again those already described under section 6.3.C.1.4.

### **6.3.C.2.3. WGLF IL-8 & The Clinical Diagnosis**

The overall assessment of the investigator of the presence or absence of active pouchitis, agreed with the levels of IL-8 in the WGLF, with some exceptions. Figure 6.21 depicts that the highest levels of IL-8 were mainly observed in the active pouchitis group (median 297.8 pg/ml) and not in the clinically normal pouches (median 37 pg/ml,  $p=0.0069$ ).

The patients with 'normal' pouches who had high WGLF IL-8 were the same as those with 'normal' pouch and high WGLF IL-1 $\beta$  (Figure 6.17, section 6.3.C.1.4), with the addition of two patients. These patients were considered initially to have a relatively normal pouch (mainly inflamed at the lower 1/2), but the biopsy characteristics altered the clinical/endoscopic diagnosis to a final diagnosis of pouchitis. The WGLF parameters were also raised (IgG: 12 $\mu$ g/ml & 20 $\mu$ g/ml, albumin: 57 $\mu$ g/ml & 16 $\mu$ g/ml, A1AT: 8 $\mu$ g/ml & 5 $\mu$ g/ml, Hb: 2  $\mu$ g/ml & 5 $\mu$ g/ml, IL-1 $\beta$ : 18.6 pg/ml & 16.7 pg/ml, respectively). A fourth patient had anal cuff inflammation, with abnormal WGLF IgG (11 $\mu$ g/ml); in retrospect the patient should have been classified under the 'other' group.

In the 'other' group, apart from those 4 mentioned already in Figure 6.17, section 6.3.C.1.4, there was one patient for whom the diagnosis had to be reconsidered after the histological result was known (i.e. pouchitis); the WGLF



**Figure 6.21.** WGLF IL-8 in the various groups according to the investigator’s assessment of active or non-active pouchitis. Patients with pouchitis had higher levels of WGLF IL-8 as compared to the patients with normal pouches.

**Table 6.13.** The WGLF parameters in the two patients in the ‘other’ group.

<b>Patients’ Diagnosis</b>	<b>IgG μg/ml</b>	<b>Albumin μg/ml</b>	<b>A1AT μg/ml</b>	<b>Hb μg/ml</b>	<b>IL-1β pg/ml</b>	<b>GE μkat/l</b>
1. Pouchitis	14	54	10	5	24.7	0.176
2. Pouch dysfunction	7	16	3	51	16.7	<0.039

parameters were again deranged (Table 6.13). The other patient had unexplained pouch dysfunction (patient 2). Table 6.13 gives details of their WGLF parameters.

#### **6.3.C.2.4. WGLF IL-1 $\beta$ IL-8 & The Various WGLF IgG Groups**

The WGLF IL-1 $\beta$  and IL-8 differed according to disease activity as measured by the WGLF IgG in ileoanal pouches. Details of these differences are given in Table 6.14.

#### **6.3.C.2.5. WGLF IL-8: Stratification Of Pouch Patients**

##### **6.3.C.2.5.i High & Low WGLF IL-8 groups**

Elevation of the cytokine levels in the WGLF may be independent to the gut protein loss or the gut blood loss. The patients therefore were divided into subgroups according to the cytokine levels. Most patients with high IL-8 had also had high IL-1 $\beta$ ; effectively a common separation was possible for these cytokines. The patients were divided accordingly to:

##### **A. Low cytokine group (n=42)**

IL-1 $\beta$  ranged from 0-34 pg/ml (median: 4.3 pg/ml, IQ range: 2.3- 8.1 pg/ml) and IL-8 ranged from 0-200 pg/ml (median: 44.7 pg/ml, IQ range: 27.1- 62.2 pg/ml);

##### **B. High cytokine group (n=14)**

IL-1 $\beta$  ranged from 15.4-420.3 pg/ml (median: 69.7 pg/ml, IQ range: 21.6- 94.4 pg/ml) and IL-8 ranged from 293-1000 pg/ml (median: 656 pg/ml, IQ range: 394-953 pg/ml).

Table 6.15 presents the WGLF parameters in these two groups.

**Table 6.14.** WGLF IL-1 $\beta$  and IL-8 in the low, medium and high WGLF IgG groups.  
The WGLF IL-1 $\beta$  & IL-8 were higher in the high IgG subgroup.

<b>WGLF</b>	<b>Low IgG group</b> IgG $\leq$ 4 $\mu$ g/ml n=35	<b>Medium IgG group</b> IgG 5-9 $\mu$ g/ml n=8	<b>High IgG group</b> IgG $\geq$ 10 $\mu$ g/ml n=13	<b>p value</b>
IL-1 $\beta$ pg/ml	4 (0-34)	8.3 (0.7-143)	59.8 (8.8-137)	0.0464** 0.0000 ***
IL-8 pg/ml	43 (0-184)	154 (55-907)	548 (47-1000)	0.0005 * 0.0277** 0.0000 ***

\* low versus medium WGLF IgG group

\*\* medium versus high WGLF IgG group

\*\*\* low versus high WGLF IgG group

**Table 6.15.** The WGLF parameters in the low and high WGLF cytokine subgroups.  
These parameters were higher in the high as compared to the low cytokine subgroup.

<b>WGLF parameters</b>	<b>Low cytokine</b> n=42	<b>High cytokine</b> n=14	<b>p value</b>
IgG $\mu$ g/ml	3 (1-17)	13.5 (5-69)	0.0001
Albumin $\mu$ g/ml	8 (1-28)	23.5 (7-69)	0.0001
A1AT $\mu$ g/ml	4 (1-10)	8 (3-44)	0.0002
Hb $\mu$ g/ml	2 (1-199)	7.5 (1-156)	0.0003
IgA pg/ml	44 (1-105)	102 (42-318)	0.0001
IgM pg/ml	9 (1-76)	22 (10-154)	0.0007
GE $\mu$ kat/l	<0.039 (0.039-0.0440)	0.097 (0.039-0.5110)	0.0005

The results are expressed as median (range).

Mann-Whitney test was used for comparison.



The biopsy neutrophilia and presence of chronic inflammation were greater in the high cytokine group ( $p=0.010$ , and  $p=0.026$ , respectively). The incidence of biopsy ulceration and villous atrophy were similar in the two groups. Table 6.16 summarises the clinical characteristics that appeared to be different in the low and high cytokine groups.

## **6.3D. The Luminal Neutrophil Migration**

### **6.3.D.1. WGLF Neutrophil Granulocyte Elastase**

#### **6.3.D.1.1. WGLF GE In The Various Diagnostic Groups**

Whole gut lavage can be utilised to assess neutrophil luminal migration either by cytological methodology or by measuring specific neutrophil enzyme activity such as the neutrophil granulocyte elastase. The detection limit of the elastase method is  $0.039\mu\text{kat/ml}$  (Handy et al, 1996). The WGLF GE in pouch patients ranged from  $0.039$  to  $0.5110\mu\text{kat/l}$ . There were no differences in the WGLF GE in the various groups: UC pouch patients, 'early' group, IC pouch patients and 'non IBD' group. The WGLF GE in the high IgG subgroup was not different from the one detected in the low IgG subgroup in the UC pouch patients (Figure 6.22).

#### **6.3.D.1.2. WGLF GE, PDAI, Functional & Other Parameters**

The WGLF GE correlated with the PDAI ( $r=0.41$ ,  $p=0.0056$ ), the pouchoscopy score ( $r=0.44$ ,  $p=0.0009$ ), and with the history of previous 'pouchitis-like episodes' recorded by the patients ( $r=0.40$ ,  $p=0.0022$ ).

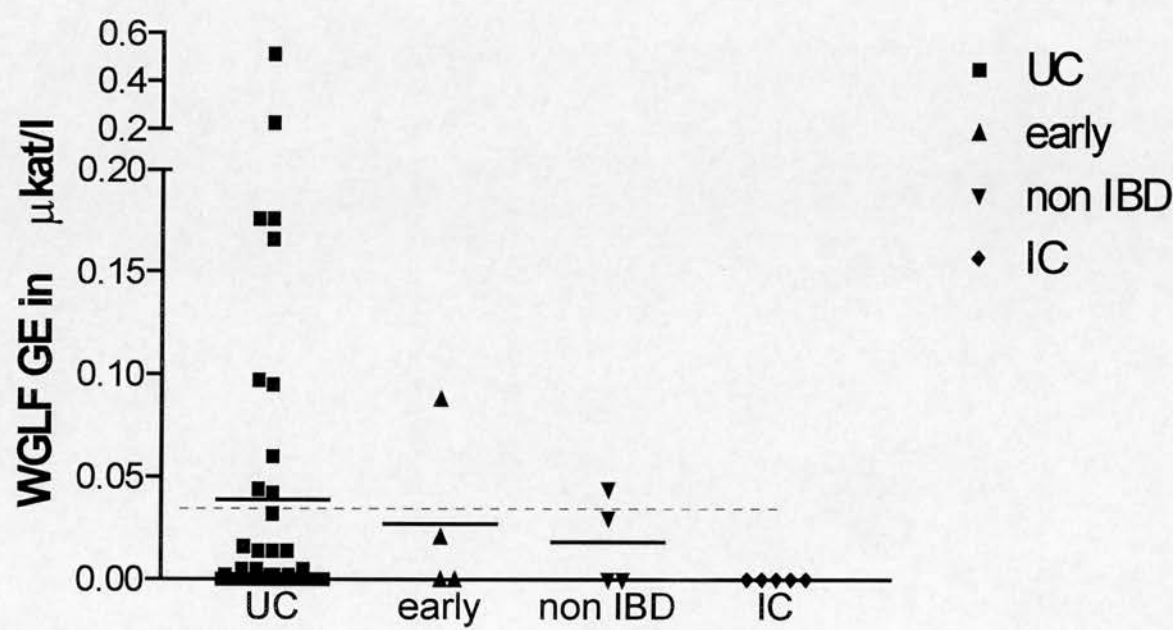
The WGLF GE related also to pouch functional parameters: the nocturnal bowel activity correlated with WGLF GE ( $r=0.44$ ,  $p=0.0010$ ).

**Table 6.16.** The clinical characteristics of the two subgroups of high and low cytokines IL-1 $\beta$  and IL-8 in the WGLF of UC pouches. These were higher in the high WGLF cytokine subgroup.

Clinical parameters	Low cytokine n=42	High cytokine n=14	p value
WCC X 10 <sup>9</sup> /l	5.8 (3.5-11.6)	10.0 (6.4-15.5)	0.0001
PLT X 10 <sup>9</sup> /l	248 (153-454)	331 (137-588)	0.0118
pouchoscopy score	2 (0-6)	3 (0-6)	0.0063
night bowel activity	1 (0-3)	1.5 (0-7)	0.0241
PDAI	3 (1-11)	6 (2-9)	0.0009

The results are expressed as median (range).

Mann-Whitney test was used for comparison.



**Figure 6.22.** WGLF GE in the various diagnostic pouch groups. The dotted line represents the detection limit of the method (0.039  $\mu\text{kat/l}$ ); the solid lines represent the mean values.

**Table 6.17** The WGLF GE in the low, medium and high WGLF IgG groups. The WGLF GE was higher in the high WGLF IgG subgroup.

<b>WGLF</b>	<b>Low IgG group</b> IgG $\leq$ 4 $\mu$ g/ml n=35	<b>Medium IgG group</b> IgG 5-9 $\mu$ g/ml n=8	<b>High IgG group</b> IgG $\geq$ 10 $\mu$ g/ml n=13	<b>p value</b>
GE $\mu$ kat/l	<0.039 (<0.039-0.079)	<0.039 (<0.039-0.042)	0.095 (<0.039-0.511)	0.0165** 0.0008***

The results are expressed as median (range).

Mann-Whitney test was used for comparison.

\*\* medium versus high WGLF IgG group

\*\*\* low versus high WGLF IgG group

**Table 6.18.** WGLF and clinical parameters in UC pouch patients with WGLF IgG<10 $\mu$ g/ml and detectable or undetectable WGLF GE. WGLF albumin, A1AT, IgA, pouchoscopy score & PDAI were higher in the GE detectable subgroup.

<b>Parameters</b>	<b>GE detectable</b> ( n=5)	<b>GE undetectable</b> (n=38)	<b>p value</b>
WGLF albumin $\mu$ g/ml	11 (9-22)	7 (1-25)	0.0379
WGLF A1AT $\mu$ g/ml	8 (5-9)	3 (1-10)	0.0071
WGLF IgA $\mu$ g/ml	62.0 (56-105)	41.5 (1-102)	0.0177
pouchoscopy score	2 (2-4)	1.5 (0-5)	0.0308
PDAI	5 (3-7)	2 (1-7)	0.0215

### 6.3.D.1.3. WGLF GE In The Various WGLF IgG Groups

The WGLF GE is presented in the three subgroups according to disease activity measured by the WGLF IgG (**low IgG**  $\leq 4\mu\text{g/ml}$ , **medium IgG**  $5\text{--}9\mu\text{g/ml}$ , **high IgG**  $\geq 10\mu\text{g/ml}$ ). See Table 6.17.

There were patients ( $n=4$ ) who had high IgG ( $\geq 10\mu\text{g/ml}$ ), but had undetectable GE ( $< 0.039\mu\text{kat/l}$ ). These patients did not seem to have any different characteristics to the rest of the high IgG patients ( $n=9$ ) who had detectable GE in the WGLF.

Patients ( $n=38$ ) with undetectable WGLF GE and WGLF IgG  $< 10\mu\text{g/ml}$  had lower albumin ( $p=0.0379$ ), A1AT ( $p=0.0071$ ), IgA ( $p=0.0177$ ) in their WGLF compared to the rest of the patients with detectable WGLF GE and WGLF IgG  $< 10\mu\text{g/ml}$ . These patients had also lower pouchoscopy score ( $p=0.0308$ ) and lower PDAI ( $p=0.0215$ ). See Table 6.18.

### 6.3.D.1.4. WGLF GE: Stratification Of Pouch Patients

#### 6.3.D.1.4.i High & Low WGLF GE Groups

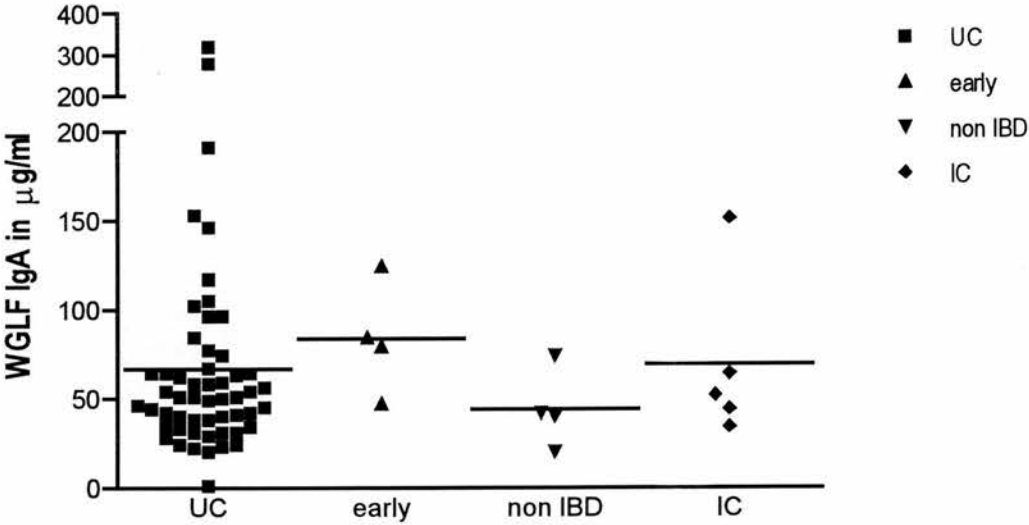
Significant luminal neutrophilia is considered with WGLF GE  $\geq 0.1\mu\text{kat/l}$  (Handy et al, 1995). This level was used to separate the patients to the high or low WGLF GE subgroups. Seven patients had WGLF GE  $\geq 0.100\mu\text{kat/l}$  ('high'). The rest 49 patients had WGLF GE  $< 0.100\mu\text{kat/l}$  ('low'). The differences in the WGLF parameters between the low and high WGLF GE groups are presented in Table 6.19. The clinical parameters in the high and low WGLF GE groups are presented also in Table 6.19.

## 6.3E WGLF Immunoglobulins In Ileoanal Pouches

### 6.3E.1. WGLF Immunoglobulin A

#### 6.3E.1.1. WGLF IgA In The Various Diagnostic Groups

Total IgA and IgM were measured in the WGLF of ileoanal pouches. WGLF IgA ranged from 1 to  $318\mu\text{g/ml}$ . There were no apparent differences



**Figure 6.23.** WGLF IgA in the various diagnostic pouch groups. The transverse lines represent the means.

**Table 6.19.** WGLF & clinical parameters in the two GE subgroups in UC pouch patients. These are higher in the high GE subgroup. Cut off point for WGLF GE: 0.1µkat/ml.

Parameters	Low GE group n=49	High GE group n=7	p value
IgG µg/ml	3 (1-69)	16 (12-33)	0.0002
Albumin µg/ml	8 (1-69)	18 (12-54)	0.0052
A1AT µg/ml	4 (1-32)	8 (4-44)	0.0035
Hb µg/ml	2 (1-199)	7 (3-30)	0.0045
IL-1β pg/ml	4.2 (0-143)	75.8 (16-137)	0.0001
IL-8 pg/ml	49.9 (0-906)	726 (394-1000)	0.0001
IgA µg/ml	46 (1-277)	146 (51-318)	0.0029
IgM µg/ml	12 (1-76)	44 (10-154)	0.0154
pouchoscopy score	2 (0-6)	3 (0-6)	0.0182
WCC X10 <sup>9</sup> /l	6.0 (3.6-15.5)	10.1 (6.4-13.3)	0.0125

between the diagnostic pouch categories (Figure 6.23). The WGLF IgA for the UC pouch group was 51 µg/ml (median; 95%CI: 42.0, 58.6 µg/ml). The WGLF IgA was similar in the 'early' group as compared to the rest of the UC pouch patients. However, the WGLF IgA in the 'early' group was higher compared to the WGLF IgA in the 'combined' (IgG < 10 µg/ml) WGLF IgG group of UC patients ( $p=0.0345$ ).

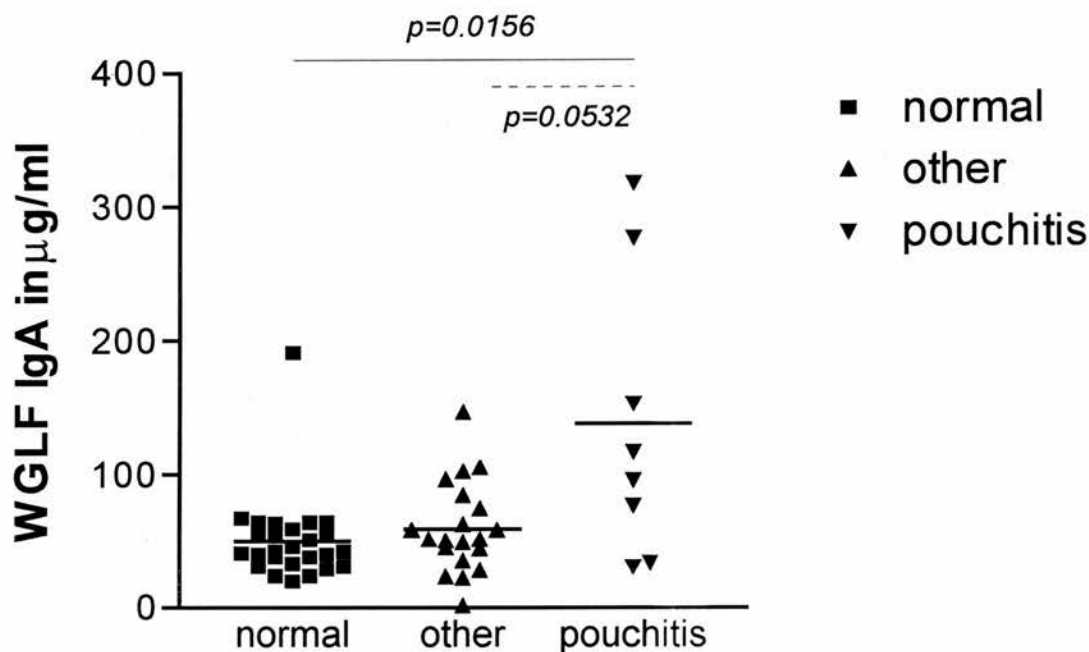
One patient had very low WGLF IgA (1 µg/ml). This was an elderly male with previous ulcerative pancolitis and a past history of alcoholic hepatitis. After the creation of his pouch he had two episodes of pouchitis, but at the time of the study he had good pouch function and no evidence of inflammation at either pouchoscopy or biopsy. The WGLF IgM was also low (1 µg/ml), but the serum levels were within normal limits (IgA 2.3, IgG 13.5, IgM 0.45 g/l). The rest of the WGLF parameters were not raised (IgG 1 µg/ml, albumin 8 µg/ml, A1AT 2 µg/ml, Hb 1 µg/ml, IL-1 $\beta$  2.3 pg/ml, IL-8 22.6 pg/ml, GE < 0.039 µkat/l).

#### **6.3E.1.2 WGLF IgA, Functional Parameters & Clinical Diagnosis**

The WGLF total IgA correlated with the functional score ( $r=0.30$ ,  $p=0.0216$ ). The WGLF total IgA related to the overall investigator's assessment of patient's condition; patients with active pouchitis according to the investigator's clinical diagnosis had a raised WGLF total IgA (Figure 6.24). The outlier in the normal group is the patient mentioned before with diabetes, NSAID etc. The two outliers in the pouchitis group are patients with long standing, almost 'chronic' pouchitis.

#### **6.3E.1.3. WGLF IgA In The Various WGLF IgG Groups**

In the previous section 6.3.A.1.5.ii, the pouch patients were stratified according to the disease activity as measured by the WGLF IgG into three subgroups: low IgG ( $\leq 4$  µg/ml), medium IgG (5-9 µg/ml), high ( $\geq 10$  µg/ml). The total WGLF IgA levels in the three subgroups are presented in Table 6.20.



**Figure 6.24.** WGLF total IgA in UC pouch patients. The patients’ status at the time of the gut lavage according to the investigator’s clinical assessment. WGLF IgA was higher in pouchitis patients.

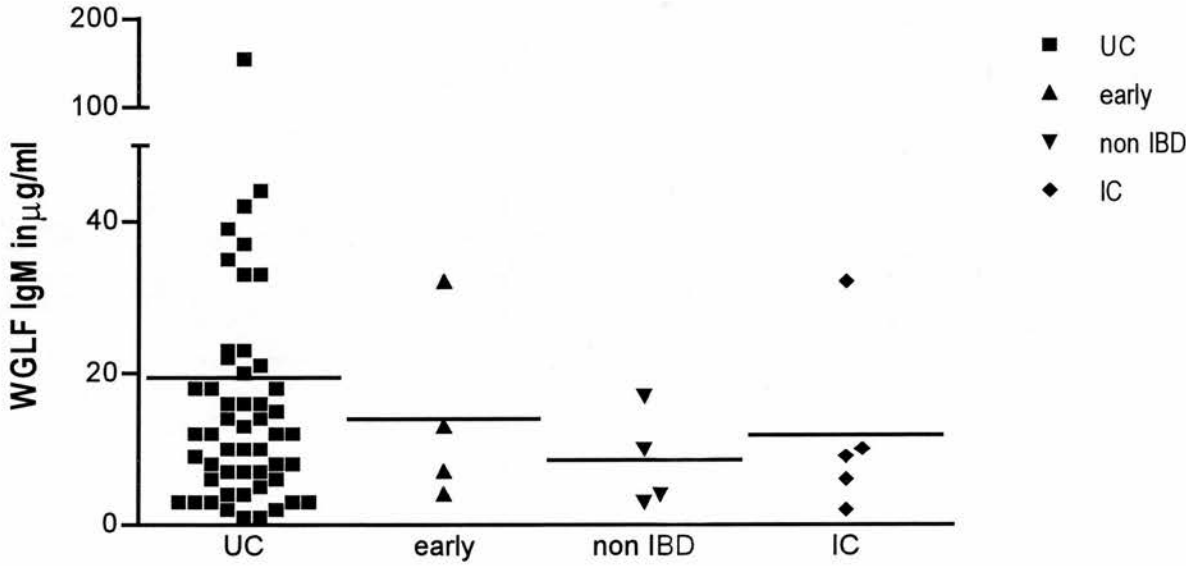
**Table 6.20.** The WGLF total IgA in the low, medium and high WGLF IgG subgroups. WGLF IgA was higher in the high IgG subgroup.

WGLF	Low IgG group IgG≤4µg/ml n=35	Medium IgG group IgG 5-9µg/ml n=8	High IgG group IgG≥10µg/ml n=13	p value
Total IgA µg/ml	43 (1-105)	50 (34-102)	77 (31-318)	0.0028*

The results are expressed as median (range). Mann-Whitney test was used for comparison.

\* low versus high WGLF IgG





**Figure 6.25.** WGLF total IgM in the various diagnostic groups. The transverse lines represent the means of the samples.

**Table 6.21.** The WGLF total IgM in the low, medium and high WGLF IgG subgroups. WGLF IgM was higher in the high IgG subgroup.

WGLF	Low IgG group IgG≤4µg/ml n=35	Medium IgG group IgG 5-9µg/ml n=8	High IgG group IgG≥10µg/ml n=13	p value
Total IgM µg/ml	8 (1-76)	16 (3-35)	21 (2-154)	0.005*

\* low versus high WGLF IgG group

**Table 6.22.** WGLF total IgM in the various clinical subgroups.

Parameters	WGLF IgM in patients with present parameter		WGLF IgM in patients with absent parameter		p value
Female sex	16 (3-59)	n=24	9 (1-154)	n=32	0.0319
Diet change	16 (1-76)	n=22	9 (1-154)	n=34	0.0330



## **6.3E.2. WGLF Immunoglobulin M**

### **6.3E.2.1. WGLF IgM In The Various Diagnostic Groups**

WGLF IgM was measured in the lavage samples of pouch patients. This ranged from 1  $\mu\text{g/ml}$  to 154  $\mu\text{g/ml}$ . The WGLF IgM for the UC group was 12 $\mu\text{g/ml}$  (median; 95%CI: 8.3,16.0). There were no differences between the various diagnostic groups (Figure 6.25). Moreover, the IgM of the 'early' group was not different from either the low or high IgG UC pouch patients, that were previously described. The outlier in the UC group is the patient with the pyoderma gangrenosum and the anal cuff inflammation that was mentioned before. He also had high WGLF IgA (318 $\mu\text{g/ml}$ ).

### **6.3E.2.2. WGLF IgM In The Various WGLF IgG Groups**

In the previous section 6.3.A.1.5.ii, the pouch patients were stratified according to the disease activity as measured by the WGLF IgG into three subgroups: low IgG ( $\leq 4\mu\text{g/ml}$ ), medium IgG (5-9 $\mu\text{g/ml}$ ), high ( $\geq 10\mu\text{g/ml}$ ). The total WGLF IgM levels in the three groups are presented in Table 6.21.

### **6.3E.2.3. WGLF IgM & Other Observations**

Women appeared to have higher levels of WGLF IgM ( $p=0.0319$ ). The villous atrophy noticed in pouch biopsy was more often noticed in a group with Patients with high WGLF IgM ( $\text{IgM} > 23\mu\text{g/ml}$ ) had more frequent villous atrophy in pouch biopsy compared with those with WGLF IgM  $< 23\mu\text{g/ml}$  ( $\chi^2=4.816$ ,  $p=0.028$ ). Patients with a change in their diet after pouch surgery had higher WGLF IgM ( $p=0.0330$ ). See Table 6.22.

## **6.3.F. Correlations Between WGLF Parameters**

Table 6.23 presents the correlations between the various WGLF parameters. WGLF IgG correlated with albumin, A1AT, WGLF IgA & IgM,

IL-1 $\beta$  and IL-8. Furthermore, WGLF IL-1 $\beta$  correlated with IL-8; WGLF IL-8 correlated with IgA. Lastly the WGLF IgA correlated with IgM.

## 6.4. CONCLUSION: WHICH TEST?

The whole gut lavage study in ileoanal pouches showed a multiplicity of parameters that relate to various aspects of inflammation and injury in pouches. In the literature, various algorithms, scores and criteria have been also described for the diagnosis of active pouchitis. The main and most strict criteria remain the Moskowitz criteria. Moskowitz criteria have been used for many years. These have been valuable in differentiating pouchitis. The recently described PDAI, combines aspects of clinical and pathological approach to the problem of pouchitis. PDAI ranges from 0 to 18, with pouchitis defined by a score of seven or greater.

Seven of the patients could be described as having pouchitis according to Moskowitz criteria. Twelve patients had pouchitis according to the PDAI. The WGLF IgG attributed increased disease activity in 13 patients (IgG > 10  $\mu$ g/ml). All the pouchitis patients according to Moskowitz criteria had PDAI  $\geq$  7. All of these patients had also WGLF IgG  $\geq$  10  $\mu$ g/ml (Figure 6.26).

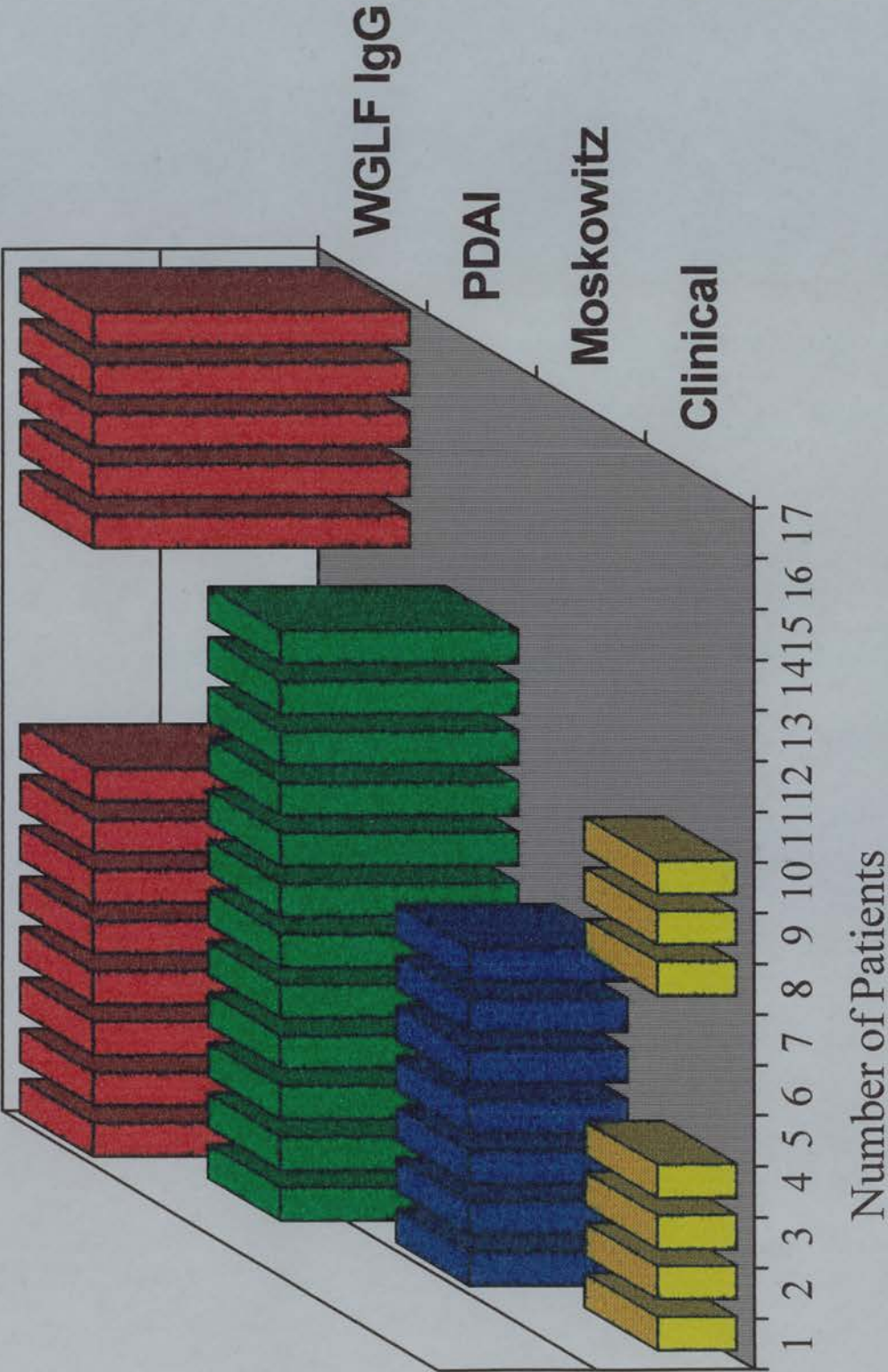
In the group of the 'normal' pouches (by global clinical assessment) two patients had to change their diagnosis to 'histological' pouchitis after the biopsy result was known. These also had raised WGLF IgG. Two other patients had high WGLF IgG, but low PDAI; these were the patients \*\*1 and \*\*4 in Table 6.4: the diabetic patient on NSAID, iron deficiency anaemia, with high systemic WCC, platelet count and ESR; and the patient with good function but severe anal cuff inflammation.

In the group that I diagnosed as 'pouchitis' (according to investigator's clinical assessment), two patients had a PDAI of 7 without a raised WGLF IgG. These patients did not fulfil the Moskowitz criteria. They were patients \*3 and \*4 in Table 6.4; one patient had pouchitis 2 months prior to the study and clinically

**Table 6.23.** WGLF parameters & correlations between WGLF parameters.

<b>WGLF Parameter A</b>	<b>WGLF Parameter B</b>	<b>r value</b>	<b>p value</b>
<b>IgG</b>	Albumin	0.72	0.0001
	A1AT	0.45	0.0004
	Hb	0.49	0.0001
	IL-1 $\beta$	0.61	0.0001
	IL-8	0.64	0.0001
	GE	0.36	0.0064
	IgA	0.61	0.0001
	IgM	0.36	0.0072
<b>Albumin</b>	A1AT	0.26	0.0464
	Hb	0.28	0.0309
	IL-1 $\beta$	0.44	0.0007
	IL-8	0.48	0.0002
	GE	0.46	0.0004
<b>A1AT</b>	IL-1 $\beta$	0.50	0.0001
	IL-8	0.55	0.0001
	GE	0.32	0.0182
	IgM	0.42	0.0015
<b>IL-1<math>\beta</math></b>	IL-8	0.66	0.0001
	GE	0.42	0.0011
	IgA	0.74	0.0001
	IgM	0.39	0.0033
<b>IL-8</b>	GE	0.66	0.0001
	IgA	0.67	0.0001
	IgM	0.49	0.0007
<b>IgA</b>	IgM	0.72	0.0001
	GE	0.28	0.0407

# Patients description according to clinical, Moskowitz, PDAI & WGLF IgG criteria





was improving still on 600 mg of metronidazole; and the second patient had anal cuff inflammation with extension of the inflammation cephalad.

A patient in the 'other' group was diagnosed to have pouchitis after the biopsy result became available. The PDAI was high and the WGLF IgG was also raised, and the patient could be categorised as pouchitis according to the Moskowitz criteria. In the same group there were two patients (\*1 and \*2 in Table 6.4) with low WGLF IgG, but had a PDAI of 7: one patient was on antipsychotic drugs; the other complained of pouch dysfunction and a month later developed pouch-vaginal fistula.

Patients in the 'other' group who had high WGLF IgG  $\geq 10\mu\text{g/ml}$ , but low PDAI were: a patient with florid anal cuff inflammation and pyoderma gangrenosum; and a patient on metronidazole who endoscopically gave the impression of pouch mucosal prolapse.

The WGLF IgG may reasonably describe the disease activity in ileoanal pouches. The discrepancy between the PDAI and the WGLF IgG might be explained from the similarities of PDAI with the other disease activity parameters like the CDAI. The WGLF IgG in combination with clinical common-sense judgement could be the 'gold standard' for categorisation of ileoanal pouches according to disease activity. Furthermore, it may be a tool of dissection and analysis of the various pouch problems.

ILEOANAL POUCHES



# **CHAPTER VII**

## **DISCUSSION**

**WGLF**

**in**

**ILEOANAL POUCHES**

## 7.1. Introduction

The hypothesis of this thesis was that inflammation of the ileoanal pouch behaves as a form of IBD. If this analogy is correct, one may expect three separate entities describing pouch inflammation as observed in IBD. First, the initiating event, the inflammatory disease activity (correlating closely with symptoms), and if analogies with Crohn's Disease are to be exact, an indolent, virtually asymptomatic destructive ulceration (Ferguson, 1996). Admittedly the latter does not seem to happen in ileoanal pouches.

Inflammation of the ileoanal pouch is a unique 'man-made' disease. It is true that the initiating event that sparks IBD is unknown. If one assumes that a form of IBD operates in ileoanal pouches, then valuable information may be gathered from the study of ileoanal pouches. This is because the time that the pouch is constructed is well known in each and every case. Accordingly a very good approximation of the time when the pouch mucosa comes in contact with the new antigenic environment can be easily identified. Thus observation of how inflammation begins in the gut from time 'zero' (time of pouch creation) till any future time is feasible. If a method of observation that is easily applicable and reliable could give information about the various facets of gut mucosal events, then a major advance in the understanding of IBD immune mechanisms will effect.

## 7.2. WGLF Parameters In The 'Early' Group

In our study we recruited five pouch patients within the first six months of pouch construction - the 'early' group. We observed that WGLF IgG and albumin - parameters indicating gut protein loss - were higher from those of the rest of UC pouch patients. Gut protein loss is a phenomenon clearly beheld in IBD, and it accurately describes disease activity (Choudari et al, 1993). The 'early' presence of such a phenomenon in ileoanal pouches may indicate that gut protein loss is an early

and non specific process. This process may be observed in the gut whenever an increase of the antigenic (?bacterial) load of the gut occurs.

In this 'early' group of pouch patients, the other whole gut lavage parameters and especially the cytokines IL-1 $\beta$  and IL-8 varied in such a way that it was difficult to conclude of any cytokine 'profile' of early pouches. Yet the number of patients recruited was small. The WGLF total IgA however was higher compared to that of pouch patients with WGLF IgG less than 10 $\mu$ g/ml. This again may be an early non specific reaction of the gut to the new increased antigenic load.

On the other hand, Apel et al (1994) has shown that in these first six months, morphological changes take place. Infiltration by neutrophils and eosinophils, a mild villous atrophy, and a progressive increase of mononuclear infiltration with lymphocytes, plasma cells, eosinophils and histiocytes being the chronic inflammatory infiltrate (Meuwissen et al 1989). With such a mucosal neutrophil activity one would have expected that luminal neutrophilia may also occur and the WGLF parameter granulocyte elastase, a marker of luminal neutrophilia, would have been invariably detectable in the lumen of early pouches. This however does not seem to be the case in the few pouches examined. This may be due to the fact that luminal and mucosal neutrophilia may not be the same phenomenon. On the other hand luminal and mucosal chemoattraction for neutrophils may be different. An alternative explanation may be based on work in our laboratory. This showed that luminal neutrophilia is a feature of active UC and active colonic CD but not of active small bowel CD (Handy et al, 1996). A large prospective study with patients having a whole gut lavage from the very beginning of pouch creation and for regular intervals afterwards will enable the study and accurate description of what happens in the lavage parameters during the initial 'adaptation' period of the first six months. This will also allow the study of mucosal immunity changes for the corresponding phases.



### 7.3. Gut Protein Loss & Disease Activity In Pouches

In the group of UC pouch patients outwith the six months period, it was shown that a certain number of ileoanal pouches exhibits gut protein loss. The latter was revealed by levels of WGLF IgG greater than 10 $\mu$ g/ml. Furthermore, there is a correlation of the protein loss as described by the WGLF IgG and the pouch disease activity parameter PDAI ( $r=0.58$ ,  $p<0.0001$ ). This relation of disease activity and gut protein loss is a feature distinctively noticeable in IBD (Choudari et al, 1993). In IBD the WGLF IgG closely portrays disease activity such as fever, anorexia, tiredness, arthropathy, diarrhoea, pain. Thus, it has been suggested that this protein losing enteropathy measures the same phenomenon as disease activity (Acciuffi et al, 1996).

The above observations are in agreement with earlier work suggesting the presence of protein losing enteropathy in pouchitis (Boerr et al, 1995). Moreover, these observations support the assumption that inflammation of the ileoanal pouch is a form of IBD. There is however the puzzling question why the agreement of WGLF IgG with the PDAI is not as good as in other forms of IBD. The WGLF IgG identified precisely all the cases who had 'active' pouchitis according to the Moskowitz criteria. The cases which did not comply the Moskowitz criteria for pouchitis and had high WGLF IgG ( $>10\mu$ g/ml) had lower levels of WGLF albumin, and less inflammatory stigmata and ulceration in their biopsies. It seems as if the Moskowitz criteria describe in an 'all or nothing' philosophy the same phenomenon as the WGLF IgG. However, some cases of inflammation (with high WGLF IgG) may not be severe enough to be included within the 'active' pouchitis group.

It is of interest that there are no large prospective studies relating the newer PDAI with the Moskowitz criteria. The original description of PDAI is based on observations on 10 patients only (Sandborn et al, 1994). There is however a study that uses the PDAI to describe disease activity in 28 pouches and relate this to faecal levels of  $\alpha$ 1-antitrypsin (Boerr et al, 1995), but no comparison is made with the Moskowitz criteria.

From a direct comparison, it seems that PDAI has close similarities with the CDAI and other disease activity indices with prominence given mainly on the symptoms and endoscopic stigmata of inflammation. For the patients with high PDAI but low WGLF IgG there was apparently an emphasis on the symptoms in three cases out of four, while on the fourth, the difference arose from biopsy from an inflamed area in continuity to anal canal inflammation. Thus, it seems that the PDAI shares the pitfalls of CDAI with the added disadvantage of biopsy sampling error. Similar observations on the limitations of the CDAI as compared to the whole gut lavage methodology have been made on patients with ileostomies, patients who are psychologically disturbed, or in children (Acciuffi et al, 1996).

If we concentrate on the cases who had high WGLF IgG and a PDAI < 7, two cases exhibited pathology consistent with gut inflammation: anal cuff inflammation. The other three cases indicated the difficulties a clinician faces when a patient has pouch dysfunction but not clearly abnormal endoscopy. Added areas of pitfalls is the difficulty to obtain good views of the entire pouch mucosa in an unprepared pouch; and the alarming patchy appearance of inflammation in some pouches (Madden et al, 1990). The whole gut lavage methodology therefore, has the advantage of offering information about the inflammatory status of the patients' gut in a 'holistic' way. This in conjunction with the clinicopathological presentation may give a common-sense approach in solving patients' problems, thus avoiding a 'therapeutic' diagnosis of pouchitis with the blind administration of metronidazole.

Another indicator that the protein losing enteropathy in ileoanal pouches is a true biological phenomenon is the observation that endoscopic stigmata of inflammation, the severity of mucosal neutrophilia and the incidence of chronic inflammation related to levels of the WGLF IgG. We have shown that in all but one patients with high levels of WGLF IgG ( $\geq 10 \mu\text{g/ml}$ ) there was invariably a high endoscopic score; the patients with severe neutrophilia and chronic inflammation had also significantly higher levels of WGLF IgG. It is of interest that metronidazole seemed to play a role in the separation of patients with severe chronic inflammation who had high or low WGLF IgG. Patients with low WGLF IgG were also on

metronidazole. This may explain the study by Madden et al (1994) where metronidazole was found to affect the diarrhoeal state of patients in chronic pouchitis, but not their histopathology or endoscopy.

The protein losing enteropathy of IgG revealed in inflamed ileoanal pouches may be a result of local production since the lympho- plasmacellular infiltrate in pouchitis is mainly composed by IgA and IgG bearing cells (Meuwissen et al, 1989). On the other hand the presence of albumin in the whole gut lavage in parallel to the IgG may indicate that the protein loss is mainly plasma derived; this is a well-established observation in IBD cases (Choudari et al, 1993).

It is of interest that another protein indicator of gut protein loss, the  $\alpha$ 1-antitrypsin, has not been as helpful in ileoanal pouches as in IBD. The levels observed were distinctly low, similar to those of ileostomies with WGLF IgG  $\geq 10\mu\text{g/ml}$ . This failure of WGLF  $\alpha$ 1-antitrypsin to relate with parameters of disease activity in our cases is in contrast to the study by Boerr on faecal  $\alpha$ 1-antitrypsin (Boerr et al, 1995). Our method of immunoturbimetry though may be less sensitive than the method of radial immunodiffusion used (Boerr et al, 1995). However, one should take in consideration that the  $\alpha$ 1-antitrypsin detection yield in faeces may vary from 2.2-97% of the amount produced by the gut at any time (Ferguson et al, 1995).

## **7.4. Gut Blood Loss In Ileoanal Pouches**

The WGLF methodology showed that there are cases with considerable bleeding in their pouches; whether this is a constant or transient phenomenon, this cannot be clearly established. The absence of relation however, with levels of systemic haemoglobin may indicate that the observed bleeding may have been of transient nature. On the other hand it was very noticeable that those cases with WGLF Hb  $\geq 5\mu\text{g/ml}$ , a level indicator of gut blood loss, had invariably raised all other WGLF parameters indicating inflammation. This therefore indicates that gut inflammation in pouches is accompanied by some blood loss.

## 7.5. Cytokines & Luminal Neutrophilia In Pouches

The presence of cytokines IL-1 $\beta$ , and IL-8 in the WGLF is in agreement with studies that showed the presence of these cytokines in mucosal pouch biopsies - especially during inflammation (Gionchetti et al, 1994). In this study, the levels of those cytokines related to the PDAI. Furthermore, the WGLF IL-8 related to the severity of mucosal neutrophilia and the chronic inflammatory response.

The systemic indices of inflammation (WCC, platelet count, CRP, ESR) related either to the cytokine profile of the pouch patients or to the protein losing enteropathy. This again has been described in IBD (Clark & Kumar, 1994).

It is difficult to speculate at present, whether the IL-8 measured in WGLF is produced mainly locally into the lumen from exudating neutrophils. Moreover, the strong relation shown between IL-8 and the neutrophil granulocyte elastase - an indicator of luminal neutrophilia - is similar to the association noticed between IL-8 and granulocyte elastase in UC but not in CD (Ghosh, 1995). This again may indicate that in pouches the neutrophil migration is a process with more similarities to the one noticed in UC than in CD. On the other hand, Teahon et al (1993) shown that neutrophils become activated in the mucosa in UC patients, whereas endoluminal chemoattractant is present in CD.

The detection of neutrophil granulocyte elastase in a number of ileoanal pouches, makes ileoanal pouches quite distinct entities. It is known that luminal neutrophilia - high levels of WGLF neutrophil granulocyte elastase - accompanies active UC, active colonic CD and it is fairly rare in active small bowel CD (Handy et al, 1996). In Chapter V, it was shown that WGLF GE is low in ileostomies with active CD, especially when compared to pouches of patients not on metronidazole. Whether this change in pattern between the ileostomy and the 'continent ileostomy', i.e. the ileoanal pouch, is due to bacterial flora and the partial colonic metaplasia observed is difficult to speculate. Future studies are required to examine the association of elastase with a marker of metaplasia such as colonic type sulphomucin.

It is however noticeable that we did not find any relation with the amount of villous atrophy and the levels of elastase in this study.

Luminal and mucosal neutrophilia have already been described with measurement of  $^{111}\text{In}$ -labelled autologous granulocytes in scans and faecal collections of patients with pouchitis (Kmiot et al, 1993). Our study however enlarges on the observation relating the phenomenon to the luminal WGLF IL-8.

## 7.6. Other Observations In WGLF In Pouches

Total IgA and IgM were measured in the WGLF of pouches and noticeably levels of IgM were the only parameter found relating to the phenomenon of villous atrophy.

The higher degree of protein loss observed in patients with 'J' pouch, even the clinically normal ones, may be related to the presence of staples in the 'J' pouches. Invariably staples extruding through the mucosa were noticed in 'J' pouches years even after pouch construction. A study indicated that 'J' pouches suffer more from pouchitis (Hewett et al, 1995). This was not seen in this study, but the differences in WGLF IgG between various pouch designs, urge a closer and more extensive appreciation of this phenomenon.

Another observation was centred on the notion that patients with high WGLF IgG invariably had tonsillectomy in the past. Though not such an association has been seen up to now with IBD, it may be worth an exploration in the future.

Finally it was noticed that the various WGLF parameters were associated between themselves, but not always strongly. In spite of the separate methodology used for each parameter (different margin error each time), and also the possibilities of uneven destruction by proteases, the close association of some of the parameters with each other is impressive. It seems that the cytokines, IgG and albumin form a close group indicating the probable relationship of the protein loss to cytokine expression. The  $\alpha 1$ -antitrypsin relates only very weakly to IL-8 and granulocyte

elastase, a clear link of this 'buffer' protein to the deleterious effect of neutrophils. The immunoglobulins on the other hand relate between themselves and also relate to the cytokines. This probably may indicate another parallel phenomenon to the one of the protein losing enteropathy.

In conclusion, WGLF parameters may be informative of disease activity in ileoanal pouches in a similar way as in IBD. One single parameter may not portray disease activity in pouchitis as in inflammatory bowel disease. However, a combination of inflammatory parameters in the WGLF may accurately characterise the mechanisms of inflammation and injury in ileoanal pouches. This will be discussed further in Chapter X.



# **CHAPTER VIII**

**NEUTROPHIL CHEMOTAXIS**

**in**

**WHOLE GUT LAVAGE FLUID**

## **8.1. INTRODUCTION**

The chemotactic signals in whole gut lavage fluid needed to be assessed in order to determine the mechanism of luminal migration of neutrophils from the gut wall. Therefore, I needed to develop a simple method that can initially be used to screen lavage samples for any chemotactic effect on neutrophils. A simple method that allows a good number of specimens to be assessed at each time is the neutrophil polarization assay (Haston and Shields, 1985). This assay is used to determine the chemotactic and neutrophil activating effect of various biological proteins in body fluids such as FMLP (N-formyl-methionyl-leucyl-phenylalanine) and IL-8. The principle of the assay relies on the fact that neutrophils floating in suspension with no chemotactic agent retain a spherical morphology. In contrast, when a chemotactic agent is present, they assume a polarized morphology. The percentage of neutrophils polarized is related quantitatively to the concentration of the chemotactic agent present.

No study has yet investigated chemotactic potential of gut derived fluid using this assay, although the polarization assay has been applied to various body fluids, especially joint synovial fluid. To utilize the method for the whole gut lavage fluid, a number of modifications had to be applied, both to the polarization assay method and to the whole gut lavage specimen processing itself. The development of these modifications along with the effect of IL-8 on the modified test and analysis of the variance of the test will be presented in this chapter.

## **8.2. MATERIALS & METHODS**

### **8.2.1. Equipment**

The basic equipment required for the polarization assay were simple.



These included:

- a) Centrifuge machine
- b) phase microscope
- c) water bath
- d) laminar flow sterile hood
- e) Neubauer counting chamber
- f) 12 ml conical centrifuge tubes with a cap (Greiner)
- g) sterile disposable pipettes (Greiner), and
- h) Phase contrast microscope with a X40 objective

## 8.2.2. Reagents

Initially sterile bottles were used for all the reagents. Later I discovered that this is not necessary as long as the media are prepared on the day of the experiment and a strict routine of non-contamination of the stock material is followed. I also followed a policy of aliquoting the reagents, to avoid deterioration during the cycles of thaw and re-freezing.

### 8.2.2A. Stock Reagents

- a. Hanks' balanced salt solution** (10X HBBS, ICN), stored sterile at 4°C.
- b. Dextran** powder average MW 150,000 (Sigma), storage at room temperature
- c. Hepes** (1M, Sigma), stored sterile at 4°C.
- d. N-formyl-methionyl-leucyl-phenylalanine** (FMLP, Sigma), prepared as 0.002M solution in DMSO and aliquoted in Epindorfs at -20°C.
- e. Dimethyl Sulphoxide** (DMSO, Sigma), stored at room temperature
- f. Heparin** mucous (Leo), stored at 4°C.
- g. Glutaraldehyde** 25% solution (BDH), stored at 4°C.
- h. PEG electrolyte solution** (Norgine), 1 sachet diluted in 1 lt. of water. The PEG solution is described as 'PEG+' when it contained the processing reagents described previously and 'PEG-' when no processing reagents were added. 'PEG+/-' was prepared from PEG solution with the addition of processing

reagents as described in the whole gut lavage processing, but without the additional newborn calf serum (NBCS).

j. **Lymphoprep<sup>TM</sup>** (Nycomed Pharma, Oslo), stored sterile in 4<sup>0</sup> C.

### **8.2.2B. Reagent Preparation**

#### **8.2.2B.a. Media**

The media preparation had to be performed just before the experiment. 25 ml of 10X stock dilution of HBBS were mixed with 5 ml Hepes and made up to 250 ml with sterile water. From this HBBS/Hepes solution, 45 ml were mixed with 5 ml of PEG+/- to give the 1/10 PEG+/- in HBBS/Hepes media.

#### **8.2.2B.b. FMLP dilutions**

On the day of the experiment 5 $\mu$ l of the stock FMLP were diluted in 4995  $\mu$ l of HBBS/Hepes media to make a 10<sup>-5</sup>M solution. Ten  $\mu$ l of the previous is then mixed with 990 $\mu$ l of media to prepare the 10<sup>-7</sup> M solution. These dilutions are 10- fold higher than those needed to make the final solutions in the test tubes.

#### **8.2.2B.c. Dextran solution**

6g of dextran powder were added into 10 ml of normal saline to give a 6% W/V solution.

#### **8.2.2B.d. Glutaraldehyde solution**

A 2.5% solution in normal saline is prepared and kept at room temperature.

### **8.2.3. Methods**

#### **8.2.3A. Cell Preparation**

Twenty ml of blood are mixed with 500 Units of Heparin (0.5 ml) and then 6 ml of the prepared blood are laid on top of 4 ml of Lymphoprep. The conical tubes are then centrifuged at room temperature for 30 min. at 200g.

Following centrifugation, the two lower layers of cells are collected: the pellet of red blood cells and the pink cloudy layer above it. With the addition of 8 ml HBBS/Hepes and 2 ml Dextran 6% for 45 min., two layers are again separated. The upper cloudy layer is collected.

The collected fluid is centrifuged at 200g for 8 minutes. The supernatant is discarded and 1 ml of sterile water is added to the pellet for 30 seconds and quickly topped with HBBS/Hepes. This step will lyse any remaining erythrocytes.

The fluid is then again centrifuged at 200g for 8 minutes and the pellet is resuspended in 0.5 ml of HBBS/Hepes.

### 8.2.3B. Initial Cell Count

Ten  $\mu$ l of the above mentioned suspension are added to 10  $\mu$ l Trypan Blue (i.e. 1:2 dilution). Ten  $\mu$ l of the above is applied onto the Neubauer chamber. Five grids are counted (a) and the total of cells available per ml are calculated as the previous total number divided by 10 (in  $10^6$  cells/ml, i.e.  $a/10 \times 10^6$  cells/ml). At this point any evidence of abnormal neutrophil morphology should lead to abandonment of the experiment.

### 8.2.3C. Preparation Of Test Tubes

The cells are dispensed into tubes containing the chemotactic agent e.g. FMLP, so as each tube may contain  $10^6$  cells.

i.e.    100 $\mu$ l of agent  
           1000/a  $\mu$ l cells  
           900-1000/a  $\mu$ l media

The agent may be media alone (for the negative control) or FMLP  $10^{-5}$  M or FMLP  $10^{-7}$  M (for the positive control). When the chemotaxis of a WGLF specimen is assessed, 100 $\mu$ l of lavage is added as an agent and the media is 900-

1000/a  $\mu$ l of HBBS/Hepes. In this case though as positive and negative control media 1/10 PEG+/- in HBBS/Hepes is substituted.

When the tubes are prepared, they are incubated for half hour in 37°C. The cells are then fixed with the gluteraldehyde 2.5% solution adding an equal volume. The cells are then washed with normal saline and centrifuged at 2000 RPM for 7 minutes. The pellet is resuspended in normal saline and the cells are washed again with normal saline and re-centrifuged. The pellet is resuspended and the tubes may be kept at 4°C for few days for further cell counting.

### **8.2.3D. Cell Microscopy & Count**

The phase microscope was used to examine the cells with the X40 objective. Any cell deviating from spherical was scored as 'polarised' and expressed as a percentage of the total. Neutrophils that were mainly spherical but had filamentous projections or blebs were counted as negative i.e. 'non polarized'. If there was a part of the cell that was ruffled and separated by a band from the smooth part, then the cell was counted as 'polarised'. 300 cells were counted per preparation tube. In all assays a negative control and a positive control were included. Pouch or IBD WGLF specimens were examined in duplicates. Negative control <5% of polarised cells was considered acceptable.

## **8.2.4. The Method Development**

Whole gut lavage has as constituents: PEG, various proteins and substances from the gut, and processing material. In theory the influence of either PEG or the processing reagents on the percentage of polarization could not be excluded, so it was decided to examine separately the behavior of the assay towards these factors.

### **8.2.4A. PEG Electrolytes**

When 'neat' PEG electrolyte solution was added to the neutrophils in the test tubes, the neutrophils did not survive. The same was true for a 1:1 dilution.

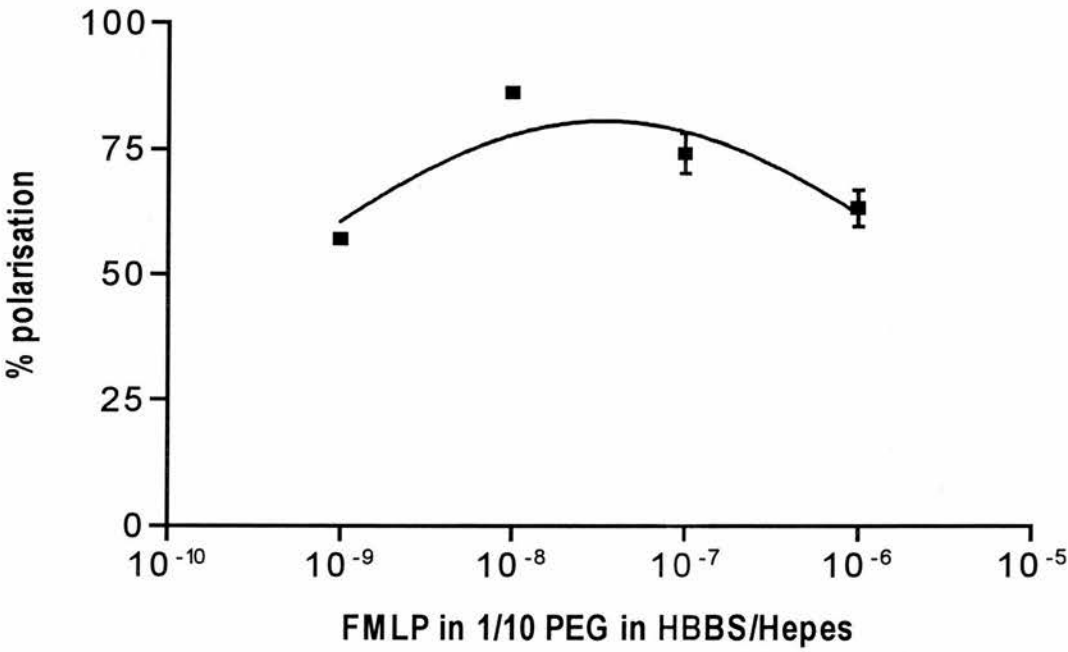
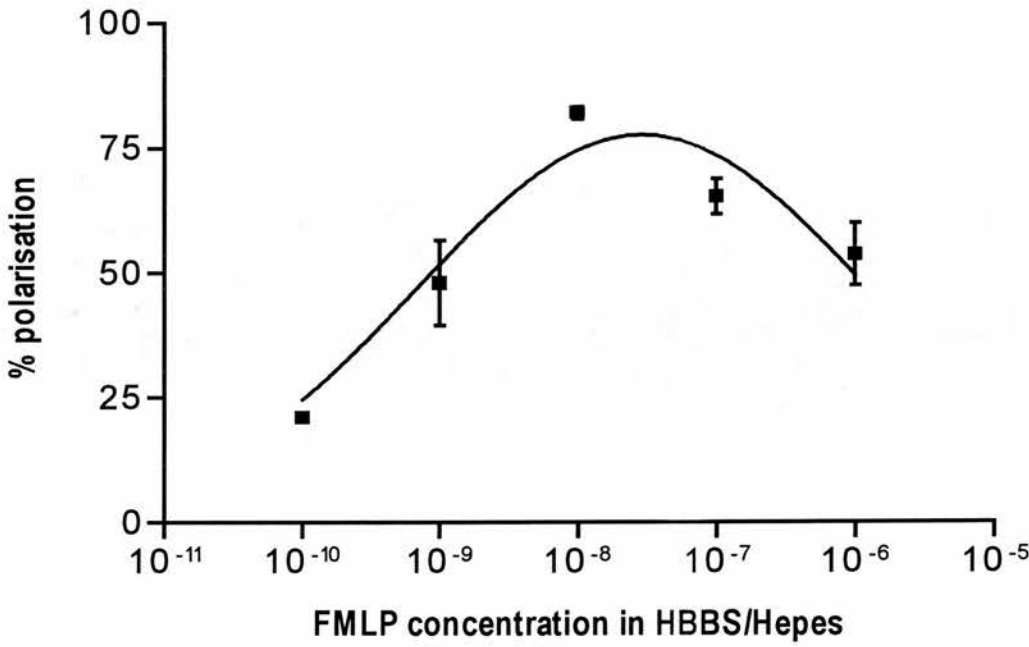
Thus a 1:10 dilution was selected as the one where the 'deleterious' effects of PEG might not affect the cells. An assumption was made, that in a 1:10 dilution there might be enough chemotactic agents to cause polarization in a lavage sample. In the original description of the assay (Haston and Shields 1985), a concentration of 1/10 of the agent is recommended for the test tube in the polarization assay. Figure 8.1 shows the FMLP dose response curve for HBBS/Hepes (n=5 experiments) and 1/10 PEG in HBBS/Hepes (n=3 experiments)

It is characteristic of the FMLP response that the  $10^{-8}$ M concentration is the most potent. This is known to happen with HBBS alone, but it seems to be so for the PEG solutions in HBBS as well. There were no significant differences between the group of values of the dose response curve in HBBS or in PEG.

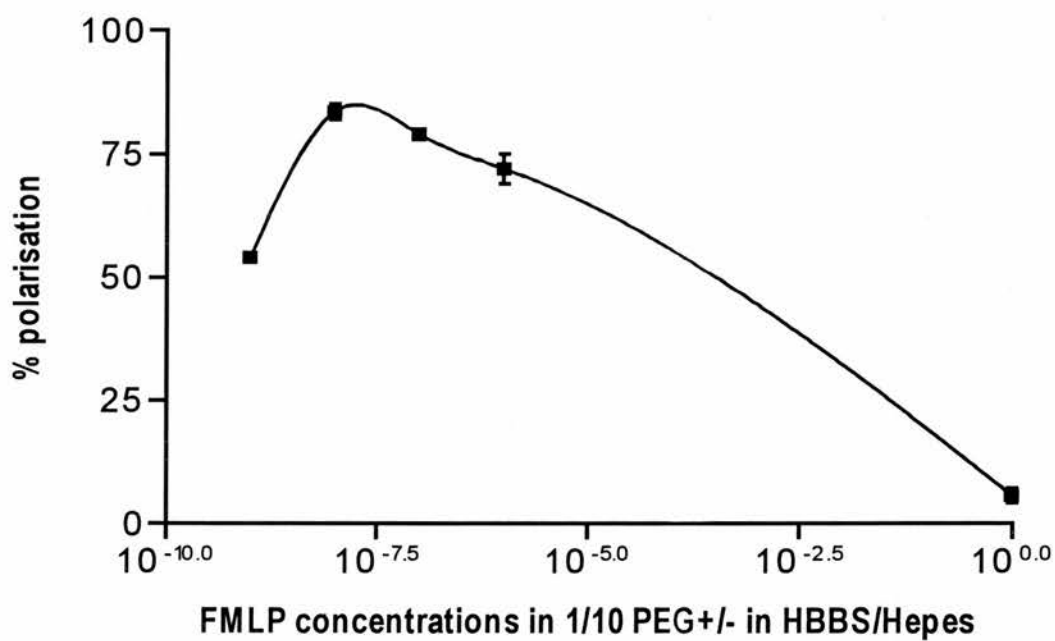
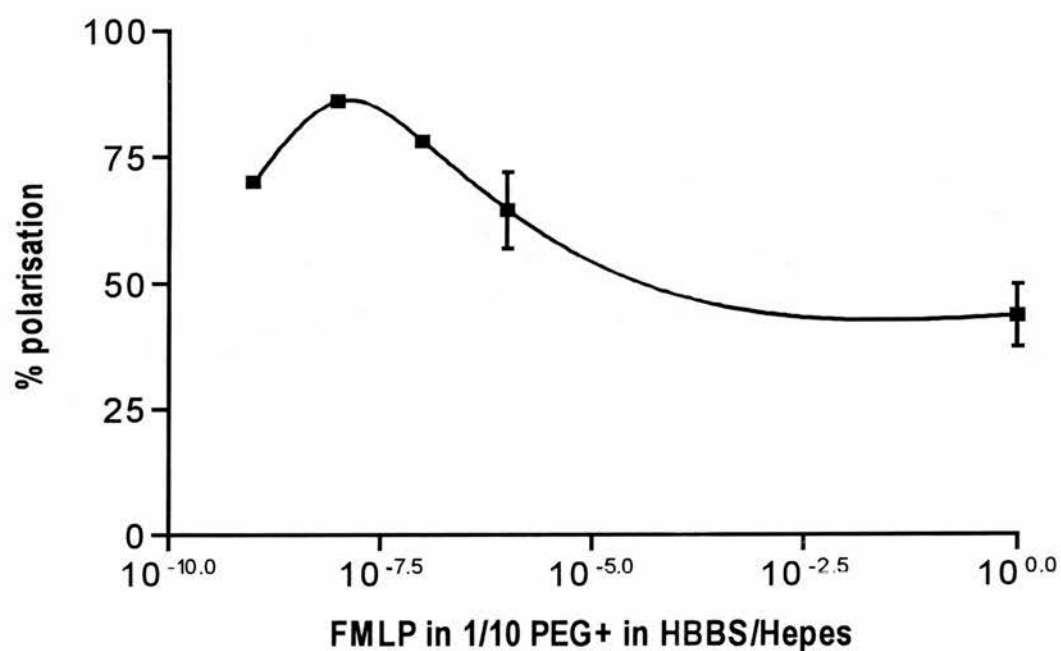
### 8.2.4B. The Processing Reagents

When the WGLF is obtained, a series of protease inhibitors is added to prevent protein breakdown. To test the influence of those on the assay, neat processed PEG (PEG+) was first assayed. As expected, the cells did not survive. Following this, a 1/10 of the PEG+ was examined. It was observed that the polarization of the neutrophils in 1/10 PEG+, without the addition of any other agent, was exceptionally high ( $p=0.011$ ). A hypothesis was formed that the substance responsible for this polarization was the newborn calf serum (NBCS). The experiment was repeated with PEG processed up to the stage of adding the NBCS, but omitting this substance (PEG+/-). The resultant dose curve was very much alike the usual FMLP curve (Figure 8.2). There were no differences in the values of the FMLP dose curve obtained in either PEG+/- media or HBBS.

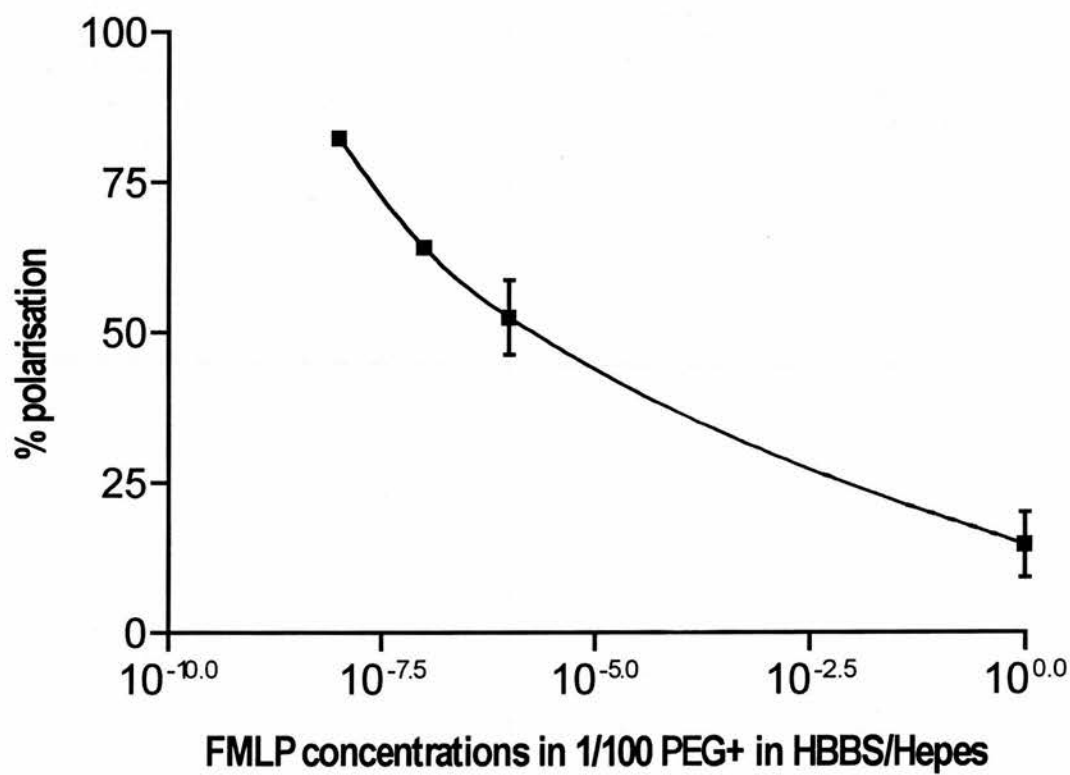
From this group of experiments (n=4 for the PEG+ and n=2 for PEG+/-) it was obvious that the standard processing of the WGLF had to be modified to use WGLF for the polarization assay. WGLF specimens with protease inhibitors without NBCS were collected and later analyzed in batches. Other experiments using 1/100 PEG+ were also performed, but still the negative control was high (Figure 8.3; n=3 experiments), though not significantly different from the HBBS controls. The option of using 1/100 dilutions of fully processed lavage samples was considered. This was not done because the sensitivity of the assay would



**Figure 8.1.** The dose response curve of FMLP in the neutrophil polarisation for (I) HBBS/Hepes and (II) 1/10 PEG in HBBS/Hepes.



**Figure 8.2.** The dose curve response for the FMLP in (I) 1/10 PEG'+ & (II) 1/10 PEG+/- in HBBS/Hepes



**Figure 8.3.** Part of the dose curve of FMLP for 1:100 PEG+ in HBBS/Hepes.



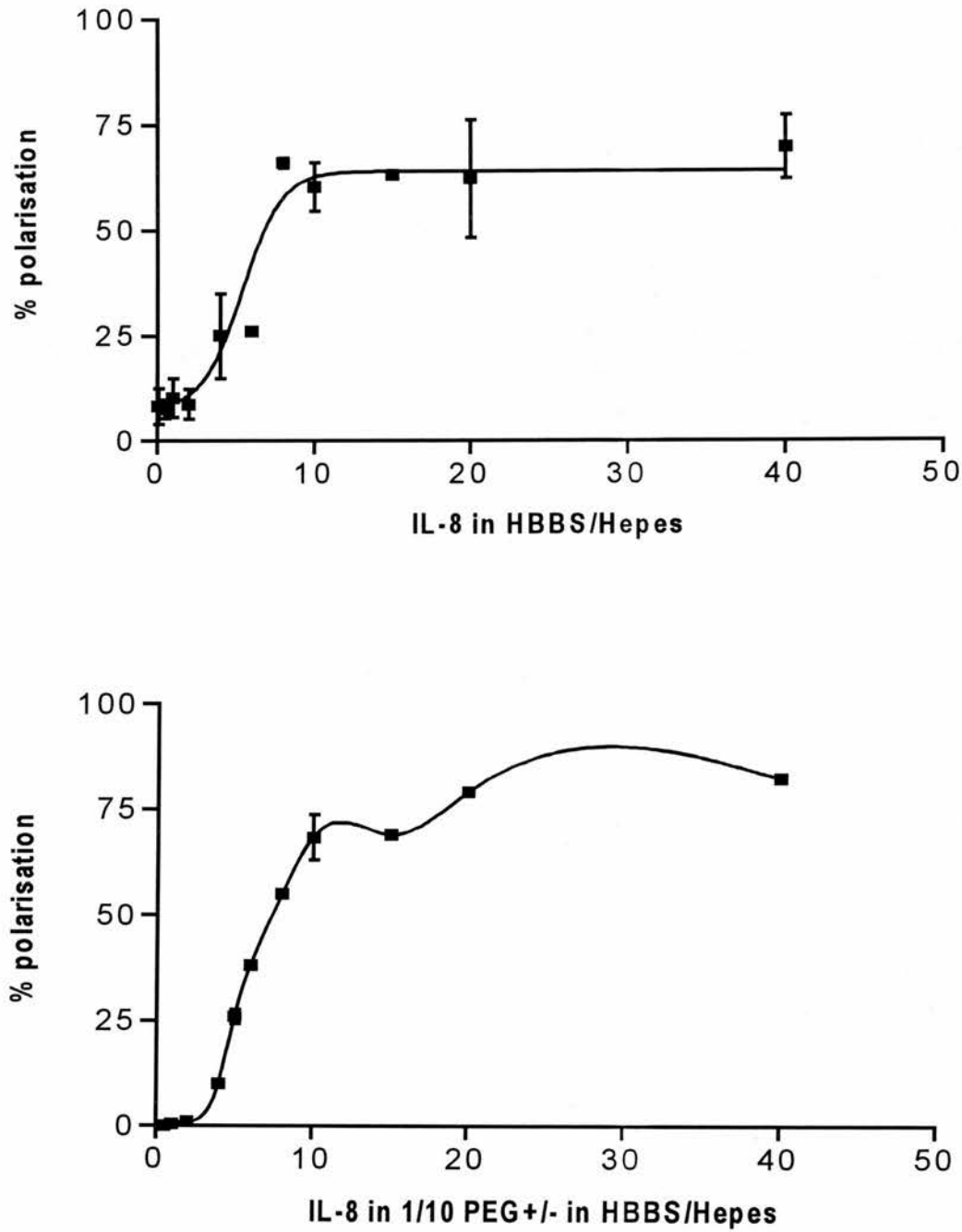
probably have been greatly reduced by excessive dilution of the possible chemotactic agents.

### 8.3. THE IL-8 EXPERIMENTS

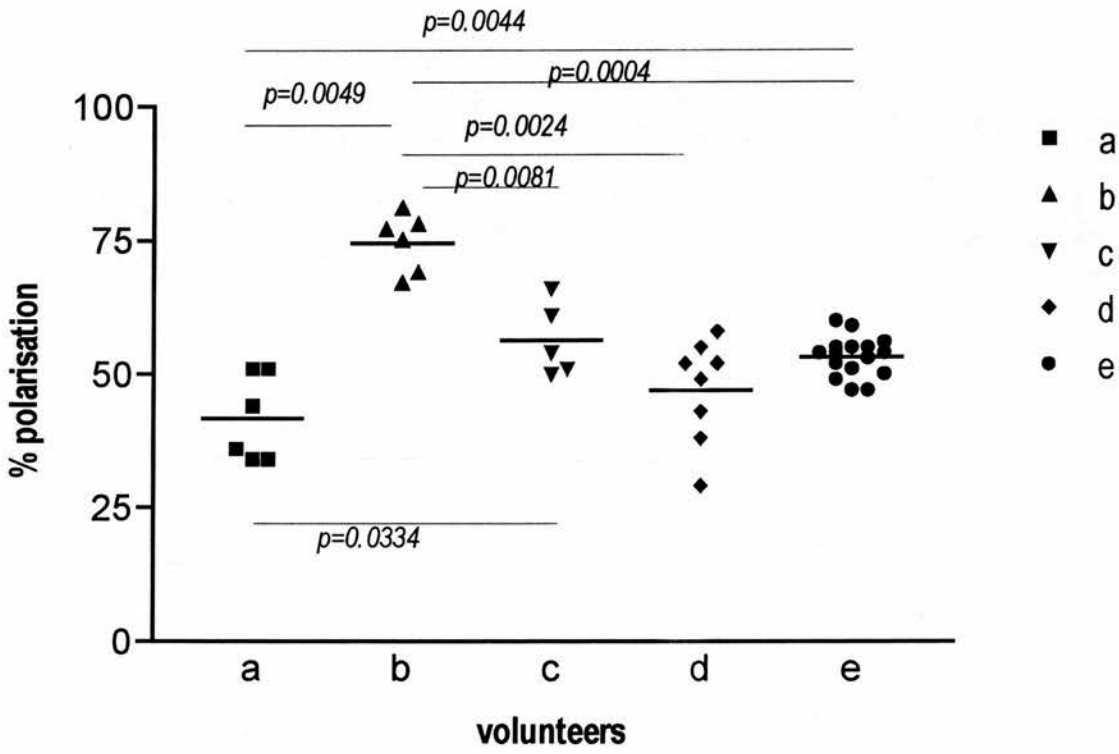
Cytokine IL-8 is one of the known chemoattractants of neutrophils. This was of particular interest to us, since IL-8 is present in the WGLF, measured by ELISA techniques (as seen in chapters V and VI). The concentrations found are up to 1000 pg/ml. It would be of interest to study the effect of IL-8 in the presence of PEG and processing reagents in relation to the polarization assay. A dose curve for IL-8 was performed using Human recombinant IL-8 purchased from R&D systems. Figure 8.4 depicts the dose response curve for IL-8 both in HBBS (n=9 experiments) and PEG+/- (n=3 experiments).

In spite of the differences, both curves seem to be sigmoid in nature, i.e. for IL-8 a concentration is reached where further increases made little difference to the percentage of the polarization.

Another observation was made at this stage. Different blood donors, in spite of having similar responses to FMLP, varied as far as the response to IL-8 is concerned. This observation meant that there are people in whom the same concentration of IL-8 will give a constantly high, medium or low % polarization of neutrophils (Figure 8.5). This difference may be due to different expression of IL-8 receptors on neutrophils in different individuals. On the other hand this observation created a difficulty in the use of the assay in whole gut lavage. It meant that, for the same lavage specimen one may get different polarization depending not only on the level of the chemotactic factors, but also on the sensitivity of the donor neutrophils to the IL-8. This hypothesis necessitated the calculation of the inter and intra assay variation, using a Quality Control (QC) sample from a pouch patient. These variations were examined against both single and multiple donors.



**Figure 8.4.** The dose response curve for the IL- 8 (ng/ml) in  
(I) HBBS/Hepes & (II) 1/10 PEG+/- in HBBS/Hepes



**Figure 8.5.** The polarisation response to 10 ng/ml IL-8 of different donors (5 neutrophil donors a,b,c,d,e)

## **8.4. THE INTER & INTRA ASSAY VARIATION AUDIT OF RESULTS**

### **8.4.1. QC Experiments - Multiple Cell Donors**

The polarisation assay is a biological assay and so the risk of non reproducibility is high. To control all possible variables as much as possible a study sheet was filled for each assay, which helped the later analysis of results.

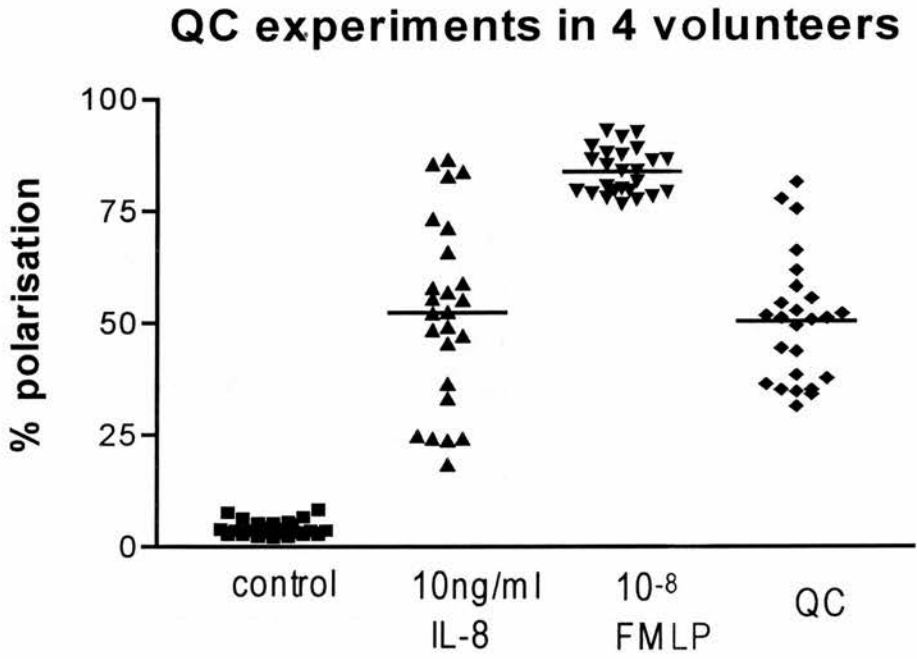
To measure the inter and intra assay variation, four cell donors were utilized each been repeated twice. Specimens were run in quadruplicates in the first set of experiments and in triplicates at the second. Agents that were tested were the 10 ng/ml of IL-8,  $10^{-8}$ M FMLP. The media used in the test tubes was the 1/10 PEG+/- in HBBS/Hepes. As negative control 1/10 PEG+/- in HBBS/Hepes was used. The QC lavage specimen was derived from the same pouch patient, been aliquoted in small Eppendorf after processing without NBCS. Figure 8.6 illustrates the distribution of the values obtained for these parameters.

The intraassay variation for QC in the above experiments was 4.8%, and for the between assay was 28% (Tijssen, 1985). The variation for FMLP was much lower, 2.2% and 5.2% respectively. These high numbers were also seen for the IL-8 (5.5% and 37.8%) and prompted further experiments of QC using one cell donor.

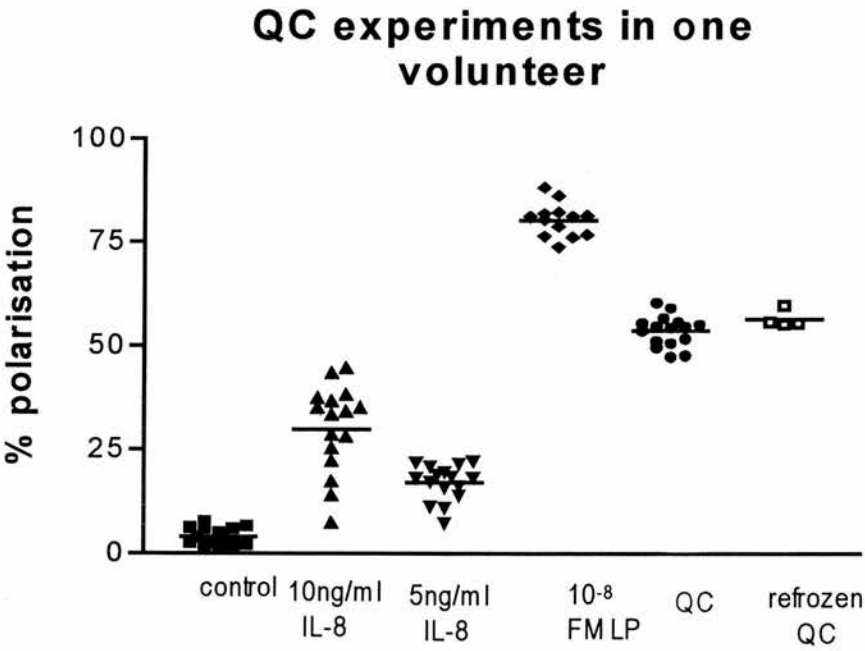
### **8.4.2. QC Experiments - One Cell Donor**

A single donor was then used for successive experiments. Figure 8.7 shows the distribution of the values obtained. The 5 ng/ml IL-8 was also assessed, along with the re-frozen QC sample, which seemed to maintained its potency.

It was noticeable that the variation for QC was much less, 4.6% and 3.6% for the within and the between assay variation. To exclude any bias in the



**Figure 8.6.** The distribution of values obtained at the 8 experiments of the QC in the multiple cell donors set



**Figure 8.7.** The distribution of values for a single cell donor at the QC experiments

analysis, further calculations were made on the results of the other donors. These showed again a 'within assay' variation of 2.5-8.1 and 'between assay' variation of 8-14.5%. The median values for the QC in either set of one or four donors, in spite of the difference in variation, were not statistically significant. On the contrary, the values for IL-8 were significantly different ( $p=0.0006$ ; Figure 8.8).

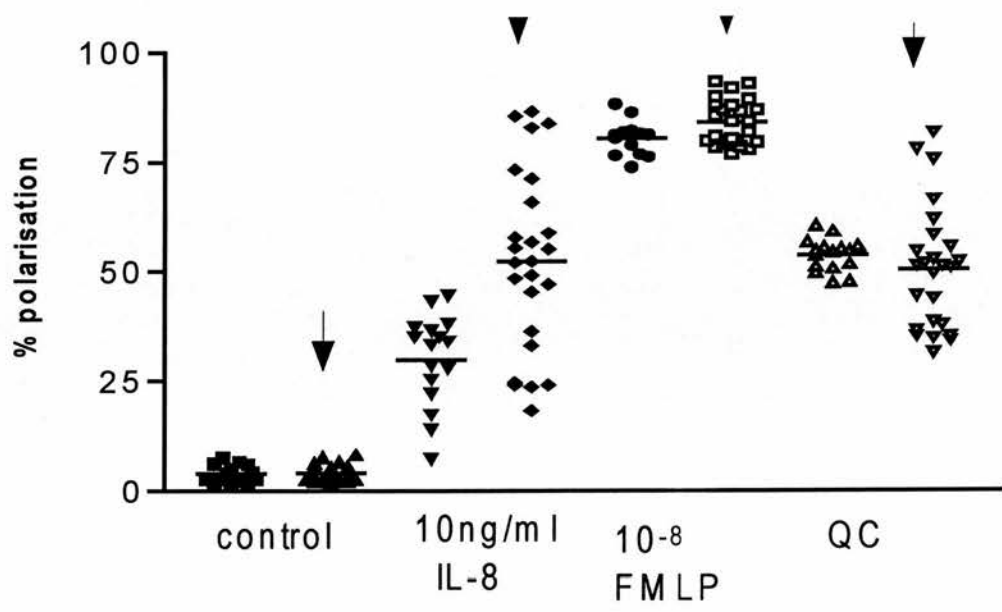
### **8.4.3. The Inter Observer Variation**

The experiments were a joint effort of two observers. There were however, a few cases that each one performed the assay individually. The scarcity of blood cell donors prevented the two observers from formally assessing the interobserver variation. On the other hand the two observers both constantly counted all cells of all the experiments and compared the results. For the QC experiments for example, the variation in the counts of QC was 8.7%. An attempt to automate counting by a computerized image analyzer proved unfruitful, since the computer could not easily recognize artifacts.

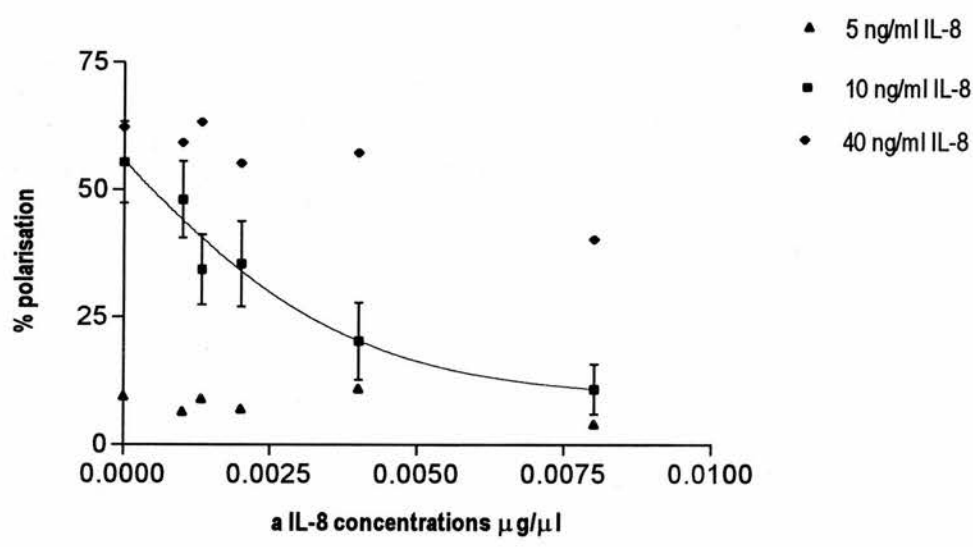
## **8.5. NEUTRALIZATION: Polarisation Assay**

### **8.5.1. Introduction**

Whole gut lavage fluid contains a variety of active substances, many of which are chemotactic; an example of one such a substance is IL-8. It would be important, to test whether the chemotactic effect of one of the constituents could be blocked, so that the effect of the other possible agents could be studied separately. For IL-8, antibodies are commercially available; but for FMLP, these have just been synthesized and were not available to us. For this reason, the effect of anti IL-8 antibodies(aIL-8) were studied alone.



**Figure 8.8.** QC experiments with the one (no arrow) & the multiple donors (arrow). IL-8 was significantly different ( $p=0.0006$ ).



**Figure 8.9.** The neutralizing effect of anti IL-8 in the polarisation by: 5 ng/ml, 10 ng/ml & 40 ng/ml of IL-8 in HBBS/Hepes media

### 8.5.2. Materials

The materials and reagents used were similar to the ones used for the main assay with the addition of the following reagents:

**a. Recombinant Human IL-8 (R&D Systems).** The 10 $\mu$ g vial was reconstituted with 1 ml normal saline with 0.1% BSA to give a 10 ng/ $\mu$ l solution. This was aliquoted and kept as stock IL-8 at -70 $^{\circ}$  C for up to 3 months. On the day of the experiment, the working dilutions were made 10 fold higher than the dilutions that are required for the test tubes.

**b. anti-human IL-8 polyclonal neutralizing antibody (R&D Systems).** It was reconstituted in PBS pH 7.4, to give a stock solution of 1 mg/ml. This remains stable for 3-6 months at -20 $^{\circ}$  C. This antibody when used in direct ELISA and Western blots shows 10% cross-reactivity with rhGRO $\alpha$ , but does not cross-react with other chemokines. It does not neutralize the biological activity of rhGRO $\alpha$ . On the day of the experiment, again 10 fold dilutions were made from stock.

### 8.5.3. Methods

The assay was performed as before with the exception that during the dextran step, an equal amount of anti IL-8 and the agent in question was incubated together in 37 $^{\circ}$  C for an hour. To the resultant mixture the media and the cells were added for the half hour incubation that is required for the polarization assay. Therefore the needed volumes and concentrations of both the agents and the anti IL-8 had to be carefully calculated before the experiment for avoidance of mishaps.



## 8.5.4. Results

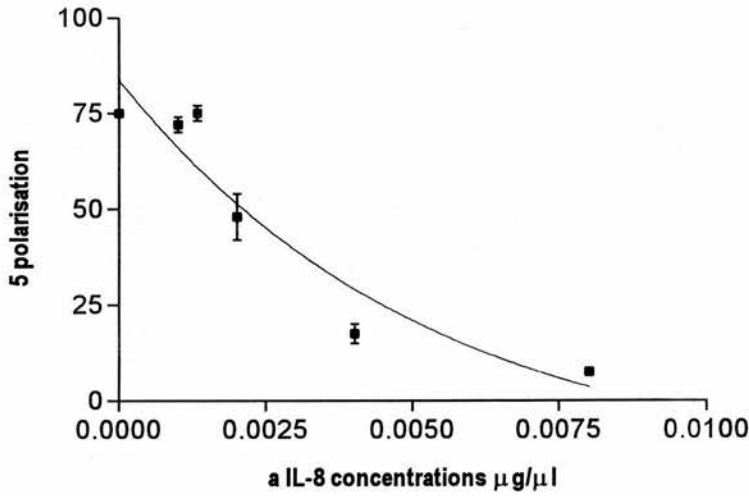
The effect of the anti IL-8 was firstly assessed in HBBS/Hepes media and using IL-8 as the main agent. It was noticed that anti IL-8 indeed was able to neutralize some of the polarizing effects of IL-8, depending on the concentration of both substances. For the dose of 10 ng/ml of IL-8 (n=6 experiments), where most of the individuals will reach their maximum plateau, the 1:125 dilution of antibody (i.e. 0.008µg/µl) was enough to almost fully abolish the polarisation response (Figure 8.9). It is noted that this dose of antibody is quadruple the dose that theoretically is required to neutralize 50% of the biological activity of the rhIL-8.

Having examined the feasibility of the experiment in HBBS/Hepes the experiment was run again with 1/10 PEG+/- in HBBS/Hepes as a medium. Only one concentration of IL-8 was checked across a variety of anti IL-8 concentrations both in HBBS and PEG+/- media (n=2 experiments; Figure 8.10). The response obtained was very similar to the one for HBBS/Hepes.

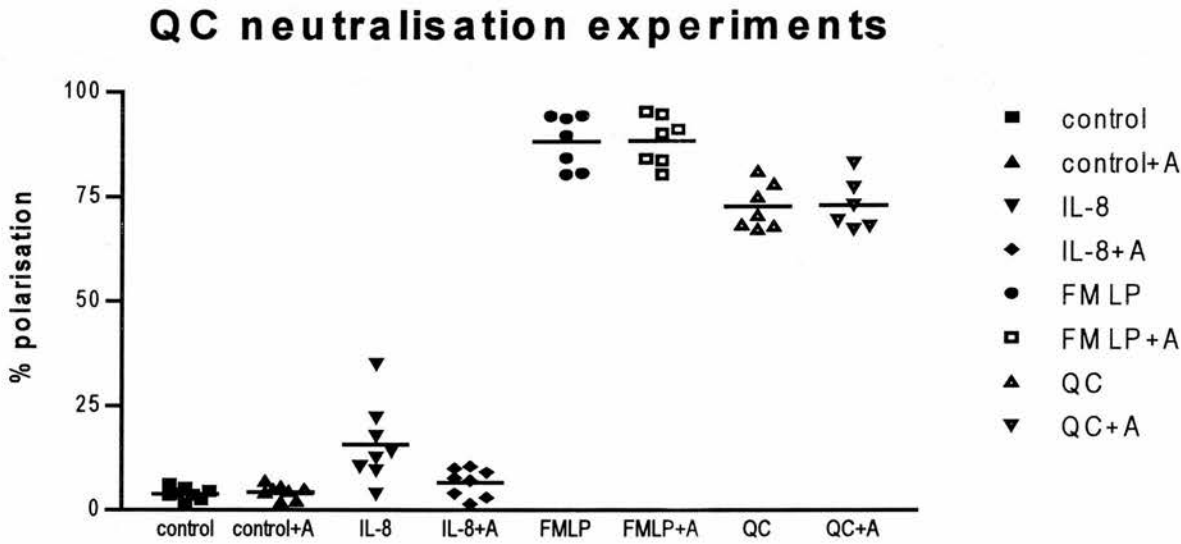
Encouraged by these preliminary results, the response of the QC sample was checked when 1:125 anti IL-8 was added. Four experiments were run in duplicates using two cell donors (Figure 8.11). The inter and intra assay variation for the QC and the QC + anti IL-8 were acceptable (2.1%, 7.4% and 1.4%, 9% respectively). As it can be seen from the graph, no change in the value of the QC was observed with the addition of the antibody. This is not surprising since the WGLF IL-8 measured in this patient was as low as 44 pg/ml.

At this point we obtained a new QC lavage from another pouch patient, which happened to have a high titre of IL-8 in his WGLF (725 pg/ml). On this occasion, the polarization of the new QC was lower than the polarization of the old QC, and becomes even lower at the addition of the antibody (Figure 8.12). These results however did not reach statistical significance.

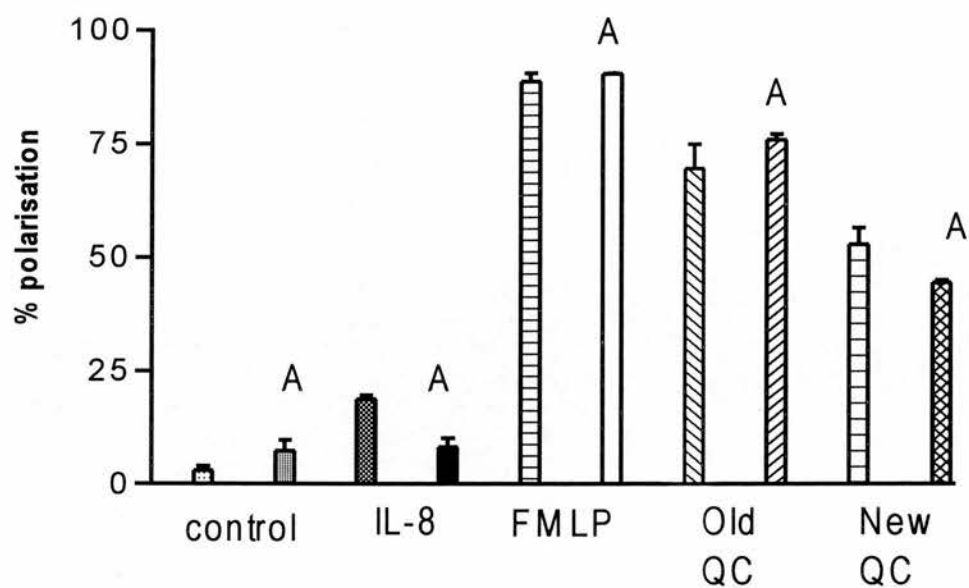
The next step was to utilize WGLF specimens collected from patients with IBD and check their polarization and its neutralization against the disease activity. Figure 8.13 depicts the percentage of polarization seen in lavage samples



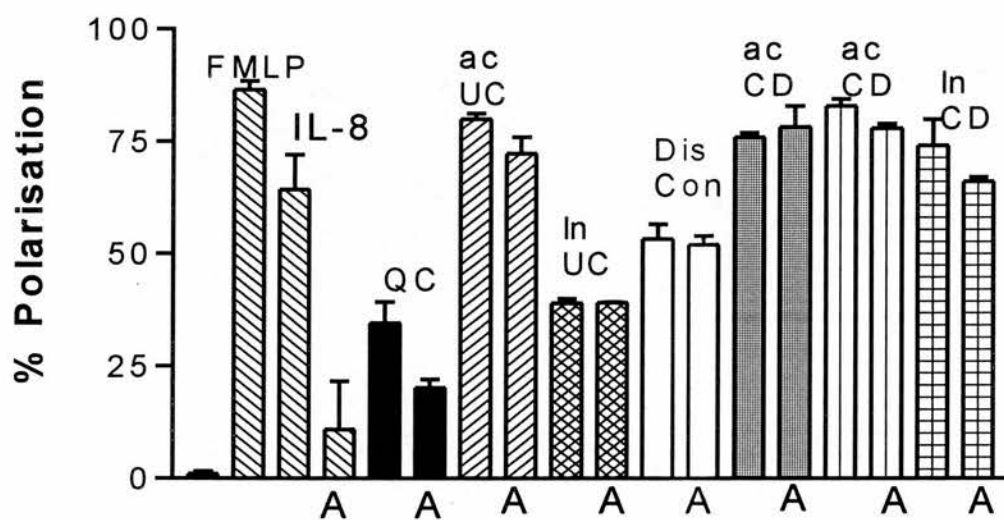
**Figure 8.10.** The dose response curve of anti IL-8 to 10 ng/ml IL-8 in PEG+/- in HBBS/Hepes



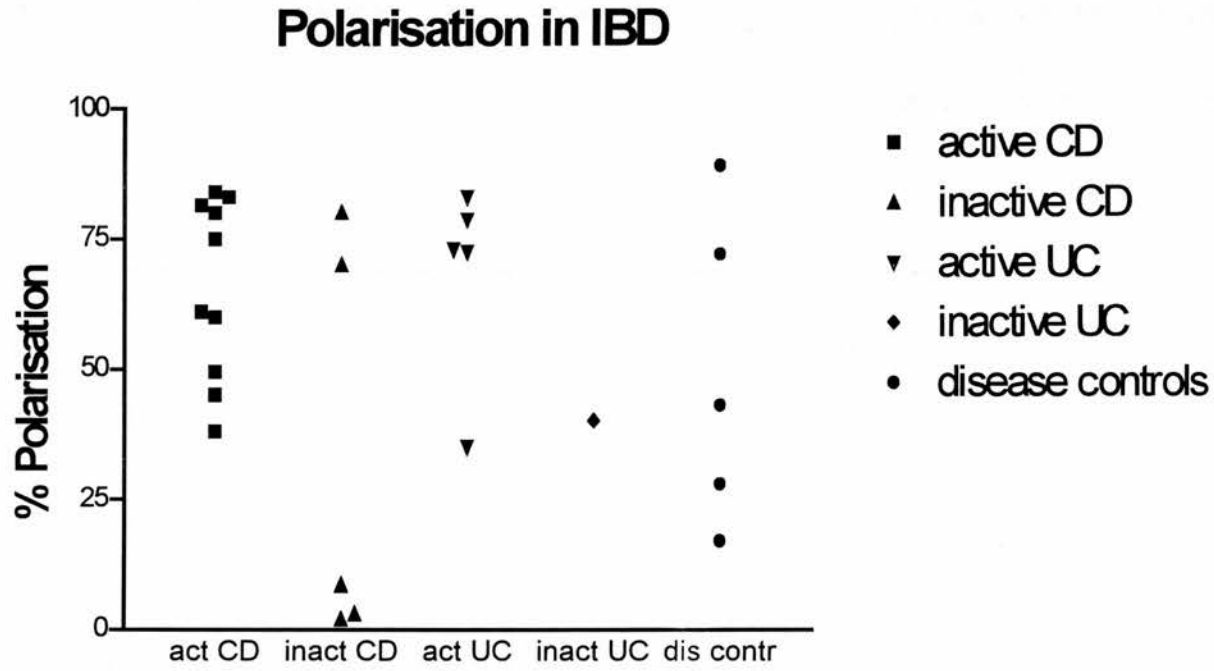
**Figure 8.11.** The % polarisation in the QC neutralisation experiments (A=anti IL-8)



**Figure 8.12.** The new QC neutralisation experiment



**Figure 8.13.** Neutralisation of polarisation in WGLF samples from IBD patients; two cell donors (A=anti IL-8)



**Figure 8.14.** Polarisation in IBD patients of varied disease activity & controls.  
Experiments in two batches, samples in duplicates.

in IBD patients. There did not seem to be an obvious pattern of behaviour relating to the clinical disease activity.

Further experiments were performed to elucidate this matter, with the inclusion of disease controls. Still however there was not a recognizable pattern of the chemotaxis of IBD patients (Figure 8.14).

The experiments of polarisation in pouch patients are described in Chapter IX, along with the discussion on polarisation experiments.



# **CHAPTER IX**

## **WGLF: NEUTROPHIL POLARISATION in ILEOANAL POUCHES**

## **9.1. INTRODUCTION**

Neutrophils migrate from the wall of the gut mucosa into the gut lumen during active mucosal inflammation. Subsequent to this migration, the neutrophils are activated in the gut lumen and degranulate. This releases noxious proteases which amplify gut inflammation and destructive ulceration. The chemotactic signal for migration of neutrophils from the blood vessels into the gut wall are well known. IL-8 plays a major role and interaction between ICAM-1 and the CD11/18a causes neutrophils to adhere to the vascular endothelium prior to migration. The chemotactic signals for migration of neutrophils from the gut wall into the gut lumen are however largely unknown. I wished to use the polarisation assay to screen whole gut lavage fluid for the presence of neutrophil chemoattractants in the lumen of the pouch.

## **9.2. PATIENTS & METHODS**

### **9.2.1. Patients**

In Chapter III, the recruitment of pouch patients is presented. The analysis of patients' clinical characteristics is given in Chapter IV. Chapter VI deals with the WGLF parameters of the obtained samples.

As explained in chapter VIII, WGLF processing without NBCS is essential to avoid high polarisation in the negative control samples. Consequently 58 pouch WGLF samples were assayed. 49 patients had UC, 4 were non IBD patients and 5 had IC. Two UC patient samples were obtained early (within 6 months of creation of the pouch). Age ranged from 18.2 to 76.8 years (median: 40.8 years). There were 30 males: 28 females. The median pouch function time was 29.8 months (range: 1.6- 66.8 months).

WGLF specimens without NBCS were analyzed from a variety of patients with intact colon. These served as controls and were used for comparison with pouch patients. Patients with intact colon included constipated patients, patients with active or inactive Crohn's disease and patients with UC. These patients had whole gut lavage as part of investigation of their GI symptoms.

### **9.2.2. Methods**

Patients consumed the polyethylene glycol electrolyte solution at a steady rate. The first clear specimen was obtained and processed omitting the NBCS step. Full description of the whole gut lavage methodology is given in Chapter III.

The WGLF samples thus obtained were analyzed with the polarisation assay following the methodology described in Chapter VIII. For the study of ileoanal pouch patients, neutrophils from a single donor were utilized and all tests were performed by the same investigator in duplicates. For the patients with intact colon two cell donors were used. The time interval between tests for each batch was one week. In each assay a negative and a positive control were included along with the QC sample, all in duplicates. The means of the duplicate samples were considered.

### **9.2.3. The Statistical Analysis**

The statistical analysis of the results used the median values; 95% confidence interval (CI), interquartile range (IQ) or range; the Pearson correlation coefficient and its p value; the non-parametric Mann-Whitney U test for unpaired comparison of observations;  $\chi^2$  with Yates correction with Odds Ratio (OR) or Relative Risk (RR). P values less than 0.05 were considered significant.



## **9.3. RESULTS**

### **9.3.1. Polarisation In Various Diagnostic Groups**

Whole gut lavage fluid samples from 58 pouch patients were assayed (Figure 9.1). The two patients who had their pouch functioning for less than six months ('early') had low % polarisation. The other groups of patients were the non IBD and indeterminate colitis pouch patients. It seems that the variation of the polarisation results is considerable. The median for the UC group is 32% with a 50% IQ range from 20% to 51%. Figure 9.2 shows the results of pouch patients as compared with patients with intact colon. It is of interest to note that the control group, i.e. patients with constipation or irritable bowel syndrome, had similar results to the ones for the main UC pouch group.

### **9.3.2. Polarisation & Clinical Parameters**

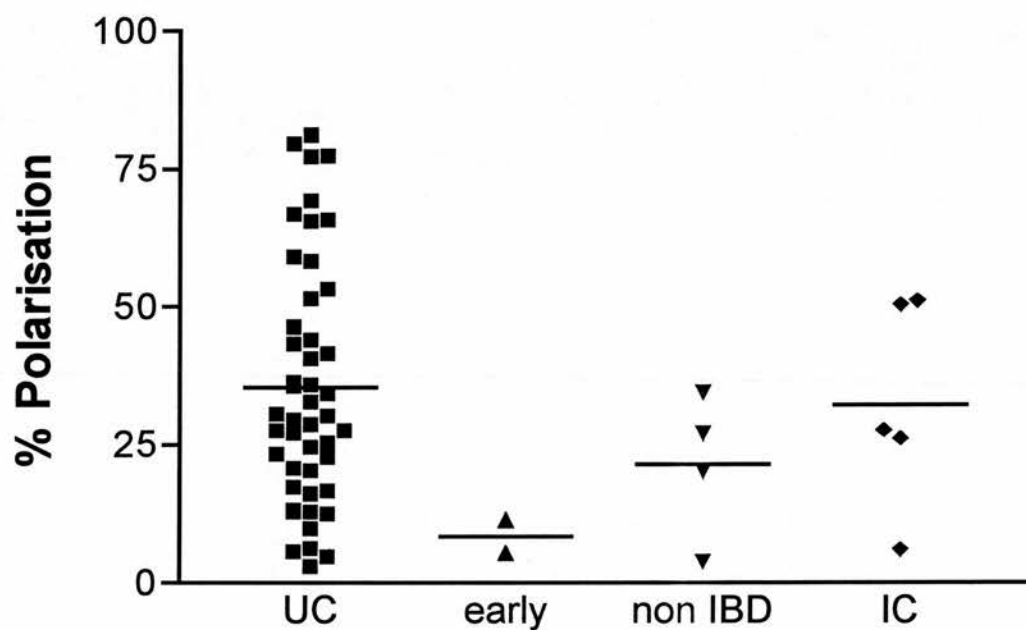
The association of the pouchoscopy score and the disease activity PDAI with the % polarisation observed for the WGLF of patients in the main UC pouch group are presented in Figures 9.3 and 9.4. There was no apparent correlation of the above scores with the % of polarisation. Patients with high pouchoscopy or PDAI scores had a variety of % polarisation.

The % of polarisation was then studied in relation to pouch biopsy characteristics and the various functional parameters, including the Öresland functional score. Yet again there were no apparent correlations.

### **9.3.3. Polarisation & WGLF Parameters**

#### **9.3.3.1. Polarisation & WGLF IgG**

The WGLF IgG appeared to correlate with disease activity in ileoanal pouches (see Chapter VI). The WGLF IgG however, did not correlate with the % of polarization (Figure 9.5).



**Figure 9.1.** The % neutrophil polarisation in the various pouch patient groups.

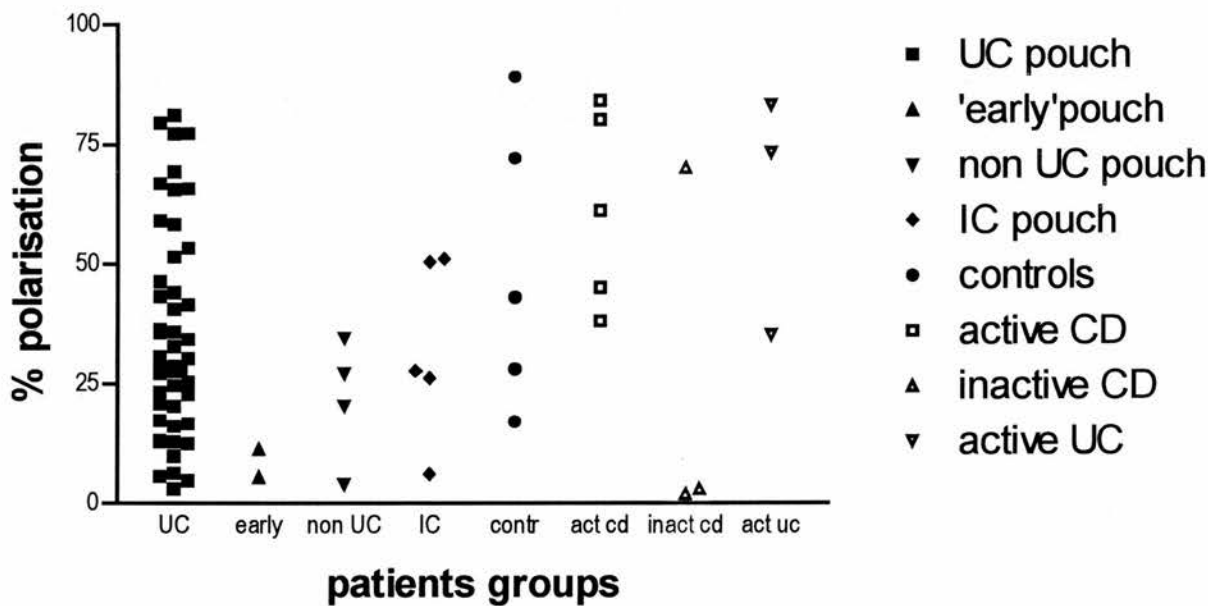
There were no significant differences between the various groups\*.

\*'UC group' - patients who underwent restorative proctocolectomy for ulcerative colitis

'early group' - patients who underwent restorative proctocolectomy for UC within the last 6 months

'IC group' - patients who underwent restorative proctocolectomy for indeterminate colitis

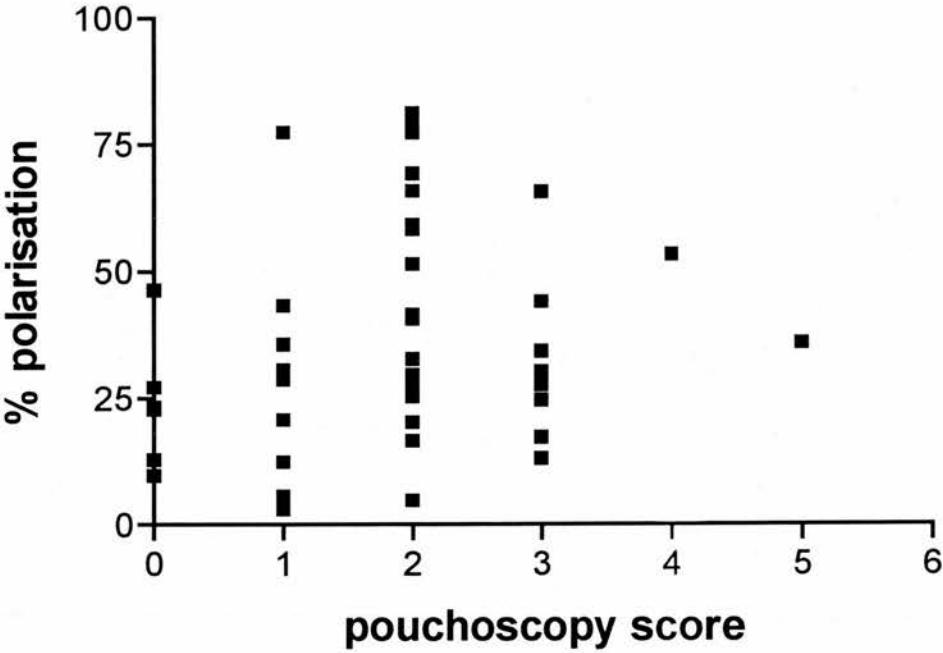
'non IBD group' - patients underwent restorative proctocolectomy for causes other than IBD



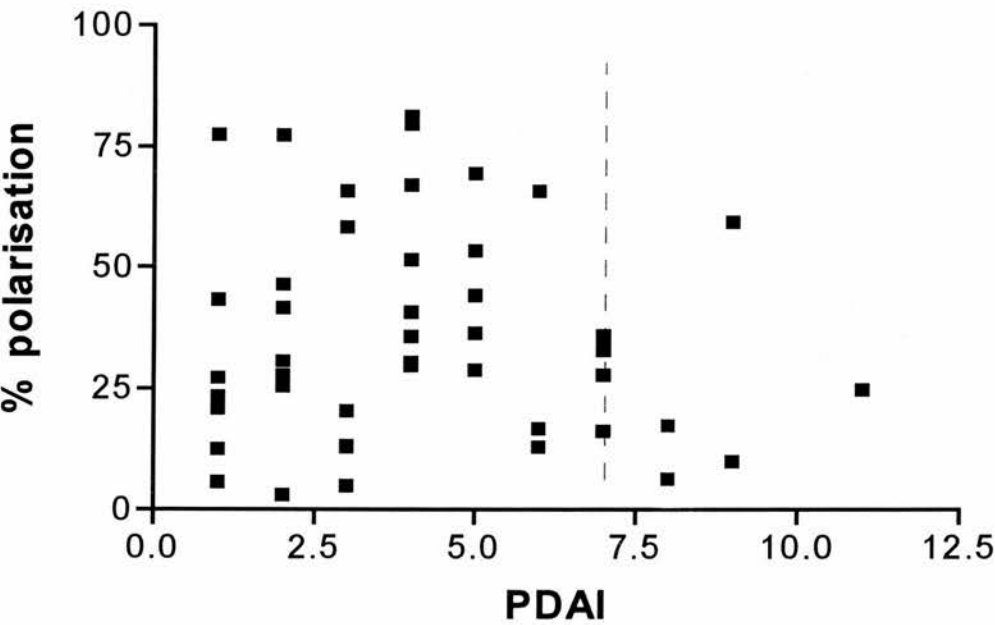
**Figure 9.2.** The % polarisation in pouch\* patients & patients with intact colon\*\*

\*‘UC group’ - patients who underwent restorative proctocolectomy for ulcerative colitis  
‘early UC group’ - patients who underwent restorative proctocolectomy for UC within the last 6 months  
‘IC group’ - patients who underwent restorative proctocolectomy for indeterminate colitis

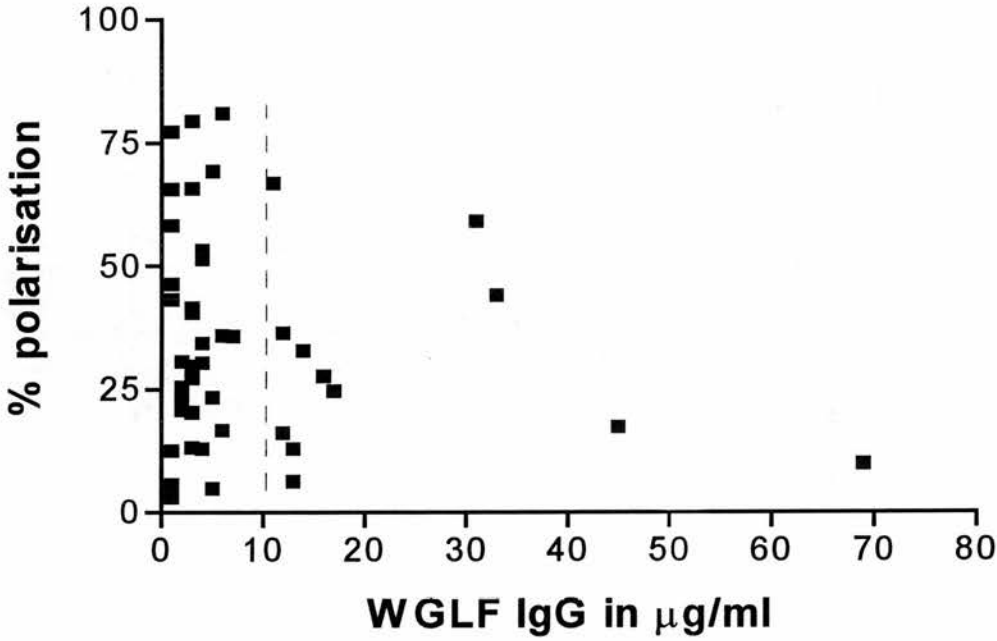
\*\*intact colon patients: disease controls  
Crohn’s disease active & inactive  
active ulcerative colitis



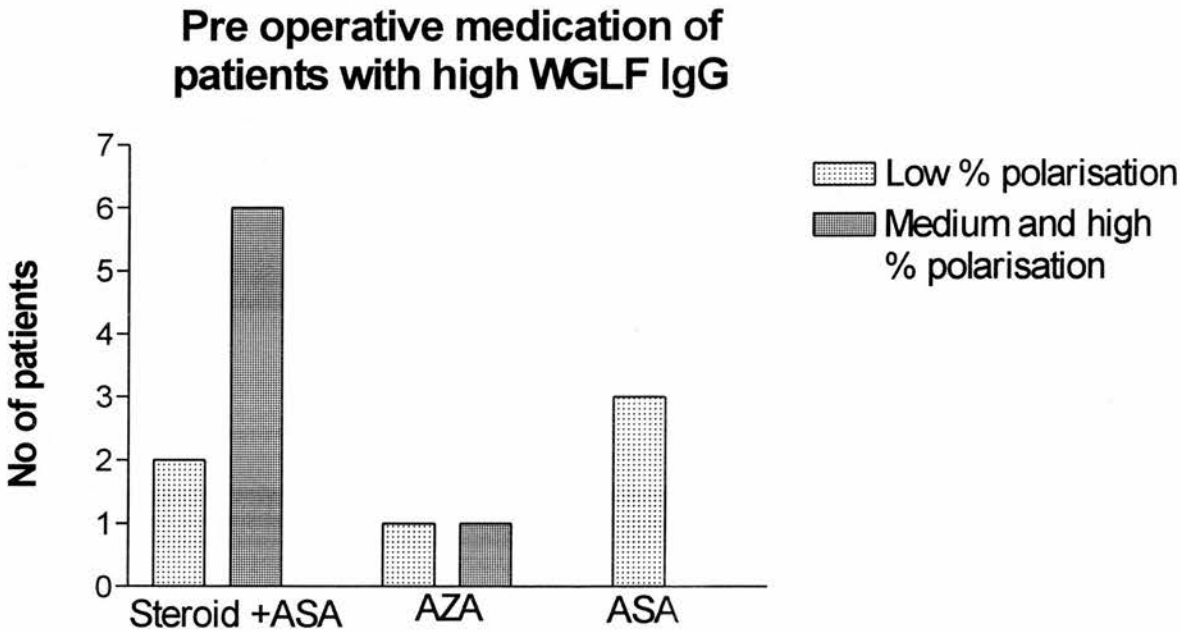
**Figure 9.3.** Pouchoscopy score & % polarisation in WGLF of UC pouch patients



**Figure 9.4.** The PDAI & % polarisation of WGLF specimens in UC pouch patients. The dotted line represents the PDAI = 7; ‘pouchitis’ is defined by PDAI $\geq$ 7



**Figure 9.5.** The WGLF IgG and % polarisation. The dotted line represents WGLF IgG= 10µg/ml; active gut inflammation is present -WGLF IgG≥10µg/ml



**Figure 9.6.** The preoperative management in the high WGLF IgG subgroup. (Steroid + ASA: systemic steroids with 5-ASA derivatives; AZA: azathioprine; ASA: 5-ASA derivatives).

Furthermore, five patients with high WGLF IgG ( $\geq 10\mu\text{g/ml}$ ) appeared to have polarisation less than 20%. These patients with high WGLF IgG and low polarisation differed in their preoperative medical management as compared with the rest of the patients ( $n=7$ ) with high WGLF IgG (see Figure 9.6).

It is evident that patients with low % polarisation and high IgG in the WGLF of ileoanal pouches, have been prescribed fewer combinations of systemic steroids and 5-ASA ( $\chi^2=4.955$ ,  $p=0.026$ ), and more often 5-ASA alone ( $\chi^2=4.955$ ,  $p=0.026$ ) as compared to the rest of the high WGLF IgG patients. This group also had more often routine colectomies performed ( $\chi^2=6.122$ ,  $p=0.014$ ).

When the biopsy characteristics of the patients with high IgG were considered, the subgroup of patients with low % polarisation had more ulceration in their pouch biopsies, when compared to the rest of the high WGLF IgG patients ( $\chi^2=5.6$ ,  $p=0.018$ ).

### 9.3.3.2. Polarisation & WGLF GE & IL-8

WGLF GE is a marker of luminal neutrophilia. One would have expected that patients with detectable WGLF GE should have high values of % polarisation if the measured % polarisation is analogous to the luminal chemotactic activity. Patients with detectable GE had a variety of values for % polarisation (see Figure 9.7). Patients with low ( $\leq 20\%$ ) polarisation and detectable GE had lower WGLF Hb ( $p=0.0412$ ).

IL-8 is detectable in WGLF (see Chapter VI & VIII) and also can cause polarisation of the donor neutrophils. There was no relation, however, of the % polarisation with the IL-8 in WGLF (see Figure 9.8).

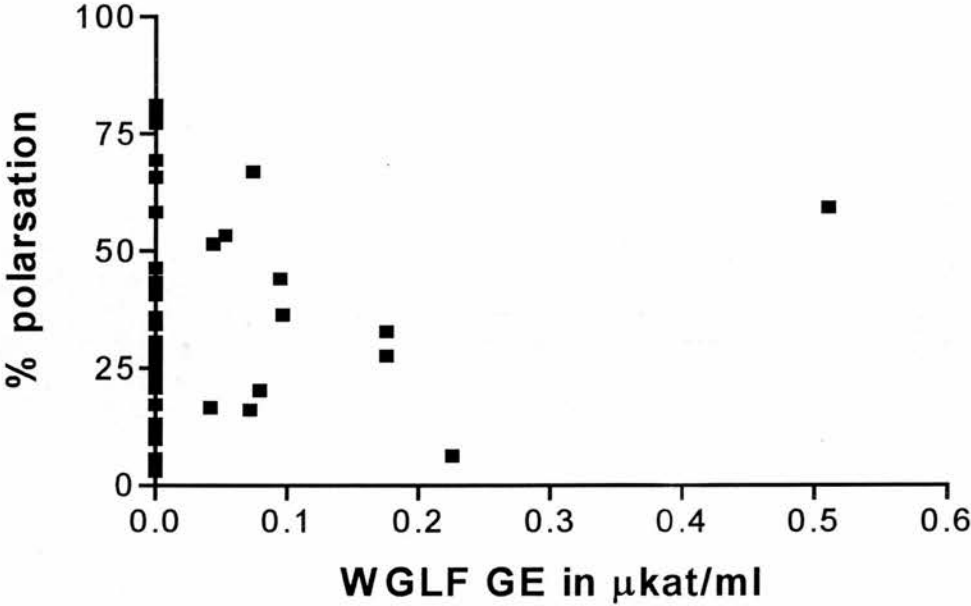


Figure 9.7. The WGLF GE and the % polarisation in UC pouch patients

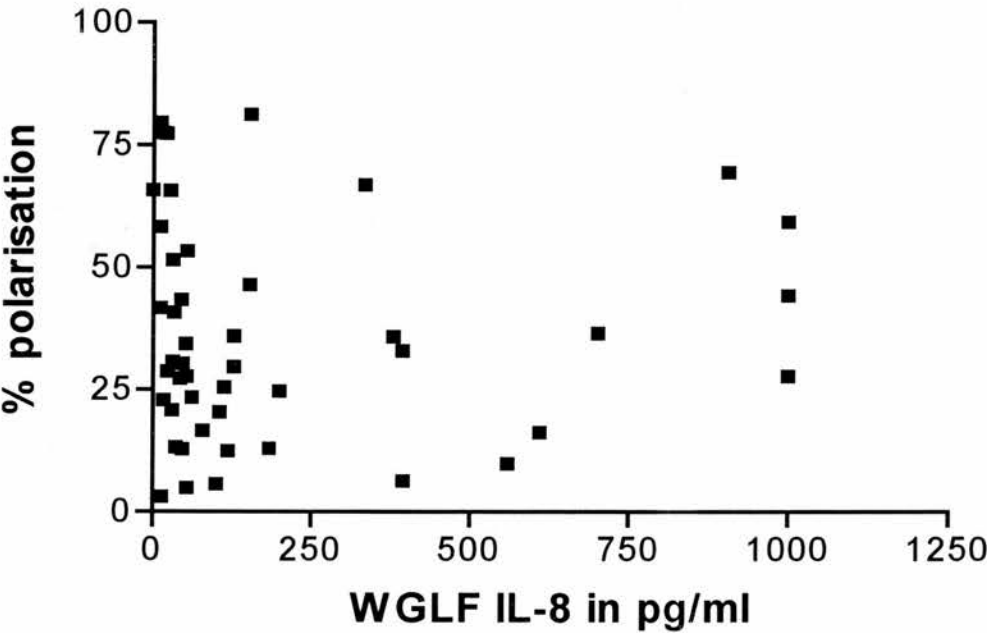


Figure 9.8. The WGLF IL-8 and % polarisation in UC pouch patients

## 9.4. DISCUSSION

The results of polarisation in control patients, i.e. patients with constipation or IBS show a great variation of % polarisation. Yet the numbers tested are small. The finding of considerable variation of % of polarisation in the WGLF of patients with ileoanal pouches may represent what may happen in 'normal' people. It may even support the concept that the luminal antigenic load present in ileoanal pouches is not fundamentally different to that of patients with intact colon. Thirdly it may indicate the presence of substances, antichemoattractants, that prevent the constant shedding of neutrophils into the gut lumen towards this chemoattractant gradient.

If we hypothesize that the % polarisation of WGLF is analogous to the luminal chemotactic drive for the patients' neutrophils during the WGLF process, then it is disappointing that there was no correlation of the % polarisation and the WGLF GE, a marker of luminal neutrophilia. On the other hand patients with detectable GE and low polarisation had low WGLF haemoglobin, compared to the rest of the patients with detectable GE. Clearly the level of WGLF Hb  $9.5\mu\text{g/ml}$  in the later indicates bleeding into the lumen (the cut off level is  $5\mu\text{g/ml}$  for our laboratory). Thus it seems that bleeding has a relation to the polarisation noticed when GE is detectable.

The disease activity parameters, and especially WGLF IgG did not correlate with the % of polarisation. For the high WGLF IgG group though, patients with low ( $\leq 20\%$ ) polarisation had more ulceration in their pouch biopsies, apart from the deference in the pre-operative medical management mentioned. Thus ulceration seems to be another influential parameter for the % of polarisation.

To try to explain all these observations two hypotheses could be made: either there is such a high inter and intra variation of the assay that results may be less meaningful; or that the assay itself gives the picture of a phenomenon which is influenced by so many parameters, that essentially it becomes independent from any other WGLF tests.



The first hypothesis is based on the fact that the polarisation assay is a biological assay. Its application on the WGLF has its difficulties as analyzed in Chapter VIII. However, we have shown in Chapter VIII that for a single cell donor, as the one used for the pouch patients, the inter and intra assay variation is acceptable. The practicality of course for using one cell donor for testing all IBD or other specimens is such that it prevents the universal application of the test for screening WGLF samples. On the other hand as a test itself has been used with success in synovial fluid, where again a multiplicity of chemotactic factors were found to be influential. In the gut however the situation may be more complicated than the synovium due to the presence of multiple bacterial species.

The second hypothesis is that the polarisation measures a phenomenon which is different from disease activity, i.e. gut protein loss. In Chapter VI also we have shown that gut protein loss and luminal neutrophilia have no strong correlation. If now the polarisation is dependent on a multiplicity of parameters of chemo and anti-chemotactic factors then it is conceivable that this polarisation assay will have no particular association with gut protein loss.

Another observation was that there was correlation of % of polarisation and WGLF IL-8, a potential candidate as a chemoattractant. In Chapter VI we have seen that WGLF contains IL-8 at a level of 0-1000 pg/ml. Calculating that for the performance of the assay a 1/10 dilution is required, then for a concentration of 1000 pg/ml the concentration present in the specimen tube will be in order of the 100 pg/ml or 0.1 ng/ml. In Chapter VIII we have shown that for this concentration the expected % polarisation is close to 0%. The values however obtained in pouch patients were significantly higher than 0%. This indicates that the IL-8 may not be a significant factor for endoluminal chemotaxis; the question however for other potential candidates is open to further research.

One possible avenue for investigation is the performance of the test with neutrophils of the patient him/herself. This may give a closer picture to reality than what we get by using neutrophils from a normal volunteer. On the other hand one may add possible antichemotactic factors and measure again the influence of those to the polarisation itself. This avenue may enable the identification of chemotactic and antichemotactic factors.



# **CHAPTER X**

## **CONCLUDING DISCUSSION**

The work in this thesis was focused on the hypothesis that inflammation of the ileoanal pouch is a form of IBD. Consequently WGLF methodology currently in use in IBD clinical practice and research would be readily applicable in ileoanal pouches.

In this thesis it has been shown that protein losing enteropathy may exist in ileoanal pouches in a similar way to what is already known in IBD. The gut protein loss correlates with disease activity and is often accompanied by bleeding, high cytokine activity and luminal neutrophilia. Accordingly estimations of WGLF protein loss along with clinical and endoscopic examination can be used for the objective assessment of inflammation in ileoanal pouches in clinical practice. The test is well tolerated, can be repeated as often as the clinical need dictates and the experience of over 1000 tests in IBD patients has shown that it gives reproducible results.

Protein losing enteropathy seems to be a feature also during the first six months of pouch function. Clinical evidence indicates that for most of the ileoanal pouches this protein loss settles after the first six months. For some patients however it resurfaces, accompanied often with luminal neutrophilia, in a fashion more similar to an ulcerative colitis inflammation.

The luminal neutrophilia relates to the luminal IL-8 measured in WGLF, showing a similar behaviour to ulcerative colitis. The chemotactic potential of the luminal contents as WGLF were assayed. However, IL-8 failed to show any association with this new WGLF parameter, the percentage of polarisation. On the other hand, it was noticed that even 'normal controls' had a variable percentage of polarisation. This observation then poses the question; which is the parameter that prevents a massive neutrophil exodus into the gut lumen for the 'normal' people who have a high percentage polarisation score.

The WGLF studies allowed characterisation and definition of pouch inflammation. This may illumine facets of inflammation in ileoanal pouches and its management. Furthermore, the studies of ileoanal pouches with whole gut lavage methodology may throw more light into the aetiology and pathophysiology of pouchitis.

An algorithm of diagnosis and management of pouchitis using the WGLF methodology is presented at the end of this chapter. If the patient presents with severe diarrhoea (>6 per day) and a high WGLF IgG and albumin, while neutrophil granulocyte elastase is detectable, then it may be assumed that the patient has either severe anal canal inflammation or active pouchitis (as defined by the Moskowitz Criteria). Pouchoscopy will help in the differential diagnosis. If however the patient has diarrhoea <6 per day with urgency, evacuation difficulties and a history of bleeding per pouch, and the WGLF shows a high IgG but relatively low albumin, then it can be presumed that the patient has 'mild' pouchitis. The patient will not fulfil the Moskowitz Criteria, but it may be argued however that treatment of the inflammatory process is necessary in this case.

Possible avenues for further research and exploration are:

1. A prospective study of WGLF measurements at regular intervals of ileoanal pouches from the time of their creation onwards. This will enlighten further some facets of inflammation during the months that follow pouch construction. It may also identify the Group C of Setti Carraro, Talbot and Nicholls (chronic inflammation and dysplasia; 1994) and give its immunological profile.
2. A prospective study to measure immune reactions before and after treatment for pouchitis. The study of cytokines or pouch permeability may reveal the presence of any prognostic factors
3. The study of other facets of inflammation, like eosinophil activity (by measuring RANTES or ECP), levels of complement and LTB<sub>4</sub>
4. The study of bacterial antigens or their antibodies (e.g. to *bacteroides*) in normal and inflamed pouches.

5. Estimation of percentage polarisation with neutrophils of the patient and with incubation of various antichemotactic factors.

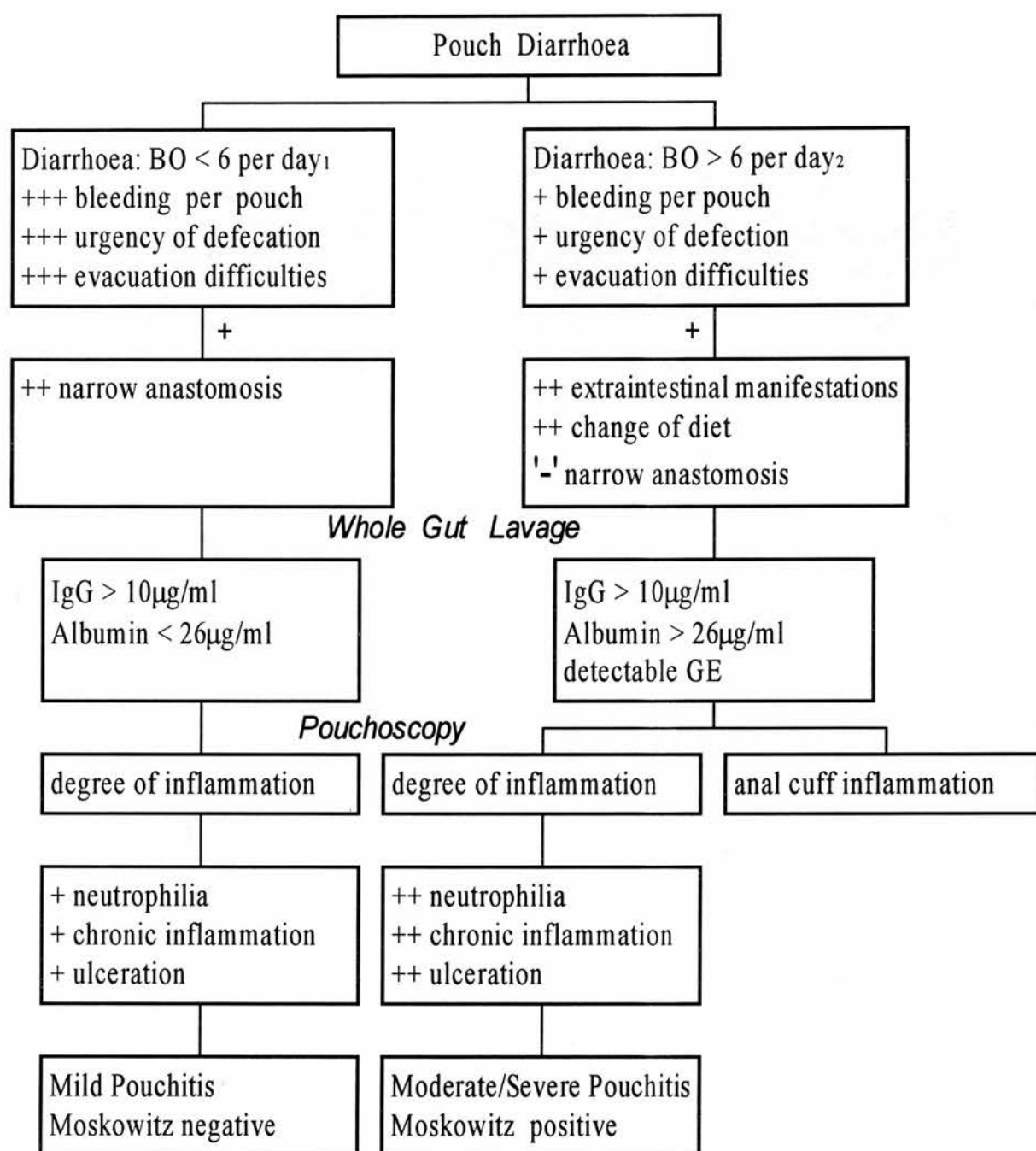
Our insights into the immunology of ileoanal pouches may only be advanced by measurements of characteristics of the disease phenomena studied.

As Lord Kelvin's dictum states,

***'... The more we measure something the more we understand it...'***



## An Algorithm of Pouchitis



1. If the patient has diarrhoea <6 per day with urgency, evacuation difficulties, a history of bleeding per pouch, and the WGLF shows a high IgG but relatively low albumin, then the patient has 'mild' pouchitis but the patient may not fulfil the Moskowitz Criteria.
2. If the patient presents with severe diarrhoea (>6 per day) and a high WGLF IgG and albumin, with detectable neutrophil granulocyte elastase, then the patient has either severe anal cuff inflammation or active pouchitis (as defined by the Moskowitz Criteria). Pouchoscopy is indicated to establish the diagnosis.

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# **APPENDICES**



## Appendix I

Information about the assays was retrieved from the Gastrointestinal Laboratory manual of the Western General Hospital. Details are given of the hazard materials and the precautions required. This information was adapted and rephrased at places for the needs of this thesis.

### 1.1 Total Immunoglobulin IgG in WGLF

#### 1.1a Principle of the Method

This Immunoglobulin is absorbed by class-specific antibodies bound to the solid phase (ELISA plate). An anti-globulin conjugate, which is enzyme labelled, then is added. This binds to the specific immunoglobulin.

#### 1.1b Reagents and standard material

- i. Carbonate/bicarbonate coating buffer 0.05M, pH 9.6 at 25°C (10 capsules in 1 litre of distilled water; Sigma C-3041). Store at 4°C
- ii. Coating antibodies: Goat anti Human IgG, Fc specific, Affinity purified (Sigma I-2136)
- iii. ELISA wash: 0.9% saline with 0.05% Tween (Polyoxyethylene-Sorbitan Monolaurate; Sigma P 1379). Store at +4°C
- iv. ELISA diluent: 0.9% saline with 0.05% Tween 20 and 1% adult bovine serum (SAPU, Law Hospital, Carlisle, Cumbria, product No S026-220)
- v. Alkaline phosphatase conjugate: Goat antihuman IgG (Sigma A-9544)
- vi. Diethanolamine liquid: Working reagent 100ml of Diethanolamine (BDH Laboratory Supplies, Poole, BH15 1TD, no 10393 4J), 0.102g Magnesium chloride ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ), 0.2g Sodium azide ( $\text{NaN}_3$ ) adjusted to pH 9.8 with 6N HCl and made up to a litre
- vii. p-nitrophenyl phosphate (Sigma 104-105 Phosphatase substrate tablets. Store at -20°C). One tablet used per 5 ml of diethanolamine working reagent (5mg/tablet)
- viii. Standard: Standard SPS 01 (Department of Immunology, PO BOX 894, Sheffield, S5 7YTUK). Store at 4°C for one month when open.



Top standard for IgG assay = 250 ng/ml

### **1.1c . Procedure and instrumentation**

The Immulon 1, flat- bottomed plates (Dynatech, UK) were coated with 1/5000 dilution of coating buffer and were stored overnight at 4°C. Then the following day the plates were washed three times with ELISA wash and then the wells were filled with ELISA diluent and incubated at 20°C for 1 hour. At the same time all standard, sample and QC (quality control) dilutions were made in ELISA diluent. The WGLF samples required 1/25 dilution. A sample from a patient (who is suspected to have raised inflammatory parameters) was used as quality control. This sample was processed and stored at -70°C. (Prior consent of the patient is required for testing for hepatitis B and HIV). Then the plates were drained, loaded with samples, standard material and QC, all in duplicate and incubated overnight at 4°C.

The following day the plates were washed three times. The conjugate was added to all wells at a dilution of 1/5000 and plates were incubated for 3 hours at room temperature. The plates were washed further for three times prior to the addition of the substrate. The plates should stand on the bench for 3 minutes prior to their placement on the shaker, with manual checking of the OD of the top standard. When the OD reached 1.0, the plate was read on the Microplate Dynatech reader MR 5000 at 405 nm. A standard curve was then made and the sample values were calculated. The correlation coefficient required was 0.985. The QC values also have to fall within 95% of the current QC mean value for the rest of the samples to be accepted.

### **1.1d Method validation**

Between batch Coefficient Variation is 12.3% at 19.8 µg/ml

## **1.2. Total Immunoglobulins IgA and IgM in WGLF**

Similar ELISA method was used for detection of these immunoglobulins in the WGLF with the exception of the standard material. The standard material for the

IgA is human IgA purified immunoglobulin from colostrum (Sigma I-2636) at top standard = 1000 ng/ml. The coating antibody used for IgA was goat antihuman IgA, a chain specific (Sigma, No I 2261), while the conjugated antibody was goat antihuman IgA, alk phos conjugated (Sigma, A3036).

Standard for the IgM ELISA again was the SPS-01 mentioned above. Top standard for IgM 1000ng/ml. As coating antibody, goat antihuman IgM was used,  $\mu$  chain specific (Sigma, No I 2386); conjugated antibody: goat antihuman IgM, alk phosph conjugated (Sigma, No A3437)

### **1.3. WGLF Albumin and $\alpha$ 1-antitrypsin ( $\alpha$ 1-AT)**

#### **1.3a Principle of the method**

When either the human albumin or the  $\alpha$ 1-antitrypsin react with an antibody at the presence of polyethylene glycol (PEG), precipitating immunocomplexes are formed that produce a turbidity, when the antibody is in excess, that can be measured by flow through spectrophotometer (PU8610-ATI Unicam, Cambridge, UK)

#### **1.3b Reagents, standards**

All reagents are analar grade and obtained from BDH unless otherwise stated.

- i. PEG 6000 reagent: 40 g of PEG 6000 (Merck, UK) with 6 g TRIS, 2 g Tween 20, and 1 g Sodium azide, adjusted with dilute HCl to pH 7.0 and made up to 1 litre with distilled water.
- ii. Anti body reagent: Dilute antihuman (goat)  $\alpha$ 1-AT (Protein Reference Unit Central Antiserum Procurement Unit, Sheffield, UK) or antihuman (sheep) albumin (SAPU S034-205) 1 in 40 with filtered PEG 6000 just prior to analysis
- iii. Standard diluent: 9g Sodium chloride, 48g PEG 3350 (Sigma, UK), and 1 g Sodium azide in 1 litre distilled water.
- iv. Standard: Serum SPS01 (Protein Reference Unit, Sheffield)

#### **1.3c Procedure**

The method required processed WGLF samples, which were centrifuged at 1500g for 10 minutes. Using a Dilugil dispenser 0.05 ml diluent, standards, and

test samples were diluted, in duplicate, into two rows of blank and tests with 0.95 ml PEG reagent in 2 ml polystyrene tubes (LIP, UK). Turbidity was read at 340 nm after 15 minutes and the standard curve was plotted after blank subtraction. Quality control used was the same as in IgG ELISA.

### **1.3d Method validation**

Repeat analysis within batch gives a coefficient of variation of 10% for  $\alpha$ 1-antitrypsin and 8.5% for albumin. Patients results are accepted only if the QC value is within the 20% range or the 17% range of the expected value accordingly.

Albumin in gut lavage is unstable prior to processing and variable amounts of 10-80% may be lost within 2 hours from the production of the specimen.  $\alpha$ 1-antitrypsin is stable in lavage for up to two hours.

## **1.4. WGLF Haemoglobin**

### **1.4a Principle of the method**

Haemoglobin is converted to fluorescing porphyrins by the removal of iron. Total haemoglobin is determined by reaction with heated oxalic acid:  $\text{FeSO}_4$  reagent which converts haem to porphyrin without loss of preformed porphyrins. A three step purification procedure eliminates other interfering fluorescent materials that may be present.

### **1.4b Reagents, standards**

All reagents are analar grade and obtained from BDH unless otherwise stated.

- i. Oxalic acid reagent: 4.0 g oxalic acid is made up to 10 ml volume with distilled water at 100°C. 0.31 g  $\text{FeSO}_4$  is then added at 100°C. 0.1 g uric acid and 0.11 g mannitol are then added with final volume 10ml, at 100°C for 5 minutes
- ii. Ethyl acetate/acetic acid 10/1 v/v (Corrosive/inflammable. Store in solvent cupboard)
- iii. 3M Potassium Acetate (294 g/l).
- iv. 3M Potassium Acetate in 1M Potassium Hydroxide (56g/l). Label Corrosive
- v. N- butanol( Rathburn Chemicals Ltd, Walkeburn). Store in solvent cupboard

- vi. 2M  $\text{H}_3\text{PO}_4$ / Acetic acid 9/1 v/v. Dissolve 68 ml of orthophosphoric acid in 300 ml distilled water (fume cupboard). Add 58 ml glacial acetic acid and make up to 500 ml with water . Label Corrosive.
- vii. Drabkins Reagent (Sigma , No 525-2): One vial contains 1 g sodium bicarbonate, 0.2 g potassium ferricyanide, and 0.05 g sodium cyanide. One vial is reconstituted to 1 litre with PEG 4000 (60 g PEG 3350, 9 g NaCl, 0.2 g sodium azide in 1 litre distilled water). Label poisonous.
- viii. Standard material Cyanomethaemoglobin: 50 mg haemoglobin (Sigma H 7379) in 100 ml Drabkins reagent. Dilute X5
- ix. Quality Control: 10 mg of haemoglobin (Sigma H 7379) and dissolve in 100ml PEG. Add sodium azide to final concentration 0.02g/100ml. Label toxic and store at  $-70^\circ\text{C}$

#### **1.4c Procedure**

The method required thawed unfiltered WGLF samples, which were then centrifuged at 2000 rpm. The supernatant was collected. 0.4 ml of oxalic acid reagent was added to 0.1 ml of WGLF supernatant, QC, haemoglobin standard, and blank (PEG) in a 10 ml stoppered quickfit tube. All tubes were heated at  $100^\circ\text{C}$  for 30 minutes. Following heating, 1 ml of the 3M potassium acetate reagent was added and then 3 ml of the ethyl acetate/acetic acid reagent were also added. The porphyrin analytes were into the upper organic phase. 2 ml of the upper organic phase were transferred to a 30 ml stoppered quickfit tube. 0.8 ml of butanol and 6 ml of 3M potassium acetate was added in 1M KOH. This removed coproporphyrin into the lower alkaline aqueous phase. The next step was to transfer 1 ml of the upper organic phase to a 10 ml stoppered quickfit centrifuge tube and add 3 ml phosphoric acid/acetic acid reagent. The top layer that contained chlorophyll was removed and fluorescence of the lower acid extract was read.

#### **1.4d Method validation**

Between batch coefficient variation is 8% for QC. Other haem containing proteins have the potential to interfere in this assay: myoglobin in meat,

peroxidases in vegetables, although neither of those should cause significant interference in lavage samples, devoid of faecal contaminants.

## **1.5. WGLF Interleukin -1 $\beta$ (IL-1 $\beta$ )**

### **1.5a Principle of the method**

A commercial, high sensitivity IL-1 $\beta$  ELISA kit was used on filtered and processed WGLF samples (Cistron Biotechnology 03-HS96). The microtitration plate was already coated with a monoclonal antibody to IL-1 $\beta$ . In the first stage samples or standards were added to the wells and incubated. The unbound IL-1 $\beta$  was then washed away. In the second stage polyclonal rabbit anti-IL-1 $\beta$  was added to the wells, so the antibody will bound specifically to the IL-1 $\beta$  bound to the solid phase. In the third stage, goat anti-rabbit IgG conjugated to horseradish peroxidase enzyme was added in order to bind any bound rabbit IgG. The fourth stage included the addition of the enzyme substrate system and by oxidation of the substrate a colour end product will appear, which was fixed with acid and then measured at 450nm. A standard curve was constructed and the concentration in IL-1 $\beta$  of unknown samples was then measured. The lower limit for detection was 4 pg/ml.

### **1.5b Procedure**

100 $\mu$ l of each standard and sample (1:2 dilution) were added into the wells and the plates were incubated for 1 hour at 37 $^{\circ}$ C. The plates were washed three times and then 100 $\mu$ l polyclonal IL-1 $\beta$  antiserum was added. The plates were incubated again at 37 $^{\circ}$ C for 20 minutes. Prior to the addition of 100  $\mu$ l conjugate, the plates were further washed three times. The plates were then left at room temperature for 20 minutes before they were again washed. 100  $\mu$ l substrate was added into the wells which were further incubated at room temperature for 20 minutes. The reaction was stopped with 50  $\mu$ l 4N sulphuric acid and the plate was read at 450 nm. The standard curve was then constructed.

## 1.6. WGLF Interleukin-8 (IL-8)

Similar ELISA method again utilising a commercial available kit (Quantikine, R&D Minneapolis USA) was used for measurement of IL-8. During the experiments it was noticed that filtration greatly reduced the amount of IL-8 available to measure. Accordingly non-filtered and processed samples were assessed. The lower limit of detection was 18 pg/ml.

## 1.7. WGLF Neutrophil Granulocyte Elastase (GE)

### 1.7a Principle of the method

The elastase activity is determined by its amidolytic effect on the substrate pyroGlu-Pro-Val-pNA. The released pNA is measured photometrically at 405 nm after the reaction has been stopped with acetic acid

### 1.7b Reagents

- i. Assay Buffer: 12.1 g Tris with 56.2 g NaCl adjusted with 1N HCl to pH 8.3 and made with distilled water up to 1 litre
- ii. Substrate S-2484 (KabiVitrum AB, Sweden): 25 mg of substrate is dissolved in 7 ml of DMSO. This is further diluted with 3 volumes of distilled water.
- iii. Acetic acid 20%
- iv. Triton X-100

### 1.7c Procedure

Unfiltered unprocessed WGLF samples were used. 1 µl Triton X-100 was added to an LP6 tube for each sample. Following this, 500 µl of lavage (defrosted samples) were added and mixed into each tube. Each sample was then sonicated (X3) at 0°C for 1 minute. 2 LP6 tubes were labeled for each sample (1 test and 1 blank) and 200 µl of assay buffer was placed in each tube at 37°C. 200 µl of sonicated samples were added to all tubes. At this stage, 200 µl of acetic acid was added to blank tubes. 200 µl of substrate was then added to all tubes and these were incubated for 3 minutes. The reaction was stopped with 200 µl acetic acid to

the test tubes. The result was read on the ELISA reader at 405 nm, and the amount was calculated as (Average test  $A_{405}$  - Average blank  $A_{405}$ ) X 2.31  $\mu$ kat/l.

#### **1.7.d. Method validation**

Between batch coefficient variation is 6% for QC. Detection limit of the method is 39 nkat/l.

### **1.8.WGLF Neutrophil Polarisation Assay**

Details of this assay are presented in chapter 8.

## **Appendix II**

### **2.1. Whole Gut Lavage Procedure Monitor Form**

The following information is recorded

1. Patient identification
2. Date of lavage
3. Quantity of Klean- Prep® in litres with time start and finish of each litre
4. Time of bowels first opened
5. Time clear specimen obtained
6. Time specimen received in the laboratory
7. Time specimen stored in freezer
8. Any comments

### **2.2. Whole Gut Lavage Patient Details Form**

The following information is recorded

1. Patient identification
2. Date of lavage
3. Lavage/serum serial number
4. Height and weight of patient
5. Diet of patient
6. Allergies
7. Smoking habit
8. Alcohol consumption
9. Indication of Whole Gut lavage



## **Appendix III**

### **Patient Questionnaire**

#### **3.1 Patient entity**

- Patient details
- Hospital and Consultant
- Date of study
- Laboratory Number
- Diagnosis
- Date of Diagnosis
- Past medical History
- Family History
- Extraintestinal manifestations if any
- Preoperative medical treatment prior to colectomy

#### **3.2 Operation Entity**

- Date of Colectomy/ restoration/ ileostomy closure if applicable
- Histology resected specimen
- Extent of disease
- Type of pouch
- Stapled or hand shown ileoanal anastomosis
- Mucosectomy or not
- Complications (dehiscence; pelvic sepsis; anastomotic stricture; peritonitis; perineal sepsis; fistula; outlet obstruction; intestinal obstruction; other)
- Extraintestinal manifestations post operative if any
- Medical treatment post operative
- Preoperative anorectal physiology studies if any

### 3.3 Function Entity

- Bowel activity day and night
- Urgency
- Evacuation difficulties
- Soiling day and night
- Use of incontinence pads day and night
- Perianal soreness
- Dietary restrictions if any
- Social handicap if any
- Bleeding
- Medication currently

Calculation of Öresland functional score

### 3.4 Pouchoscopy & Pouch Biopsy Entity

- Pouchoscopy score (1-6); presence of oedema, granularity, friability, loss of vascular pattern, mucous exudate, ulceration
- Anal cuff inflammation
- Stenosis at the ileoanal anastomosis
- Investigator's clinical diagnosis at time of lavage/ pouchoscopy
- Previous 'pouchitis' episodes according to patient and their duration
- Histopathology score (Neutrophilia, ulceration, chronic inflammation, villous atrophy)
- Calculation of PDAI/ Moskowitz criteria diagnosis

### 3.5 Test Entity

- Haemoglobin, white cell count, Platelet count
- Erythrocyte Sedimentation Rate (ESR)
- C-Reactive Protein
- Serum immunoglobulins (IgG, IgA, IgM)

- WGLF tests
  - IgG
  - Albumin
  - $\alpha$ 1-antitrypsin
  - Haemoglobin
  - Interleukins IL-1 $\beta$  and IL-8
  - Total IgA
  - Total IgM
  - Granulocyte Elastase
  - % Polarisation

## **Appendix IV**

### **Tests outside the GI Laboratory**

As referred in chapter III, the following parameters were recorded:

- a. Full Blood Count
- b. Erythrocyte Sedimentation rate (ESR)
- c. C-reactive protein (CRP)
- d. Serum Immunoglobulin plasma levels

The specimens were assayed either in the Western General Hospital or the Royal Infirmary of Edinburgh Haematology, Clinical Chemistry or Microbiology laboratories. The Full Blood Count was estimated on a Coulter Counter. The ESR was read manually. A radioimmune assay was used to measure the CRP. For serum Immunoglobulins immunoturbimetry was used. Further details are available from the manuals of the above mentioned laboratories.

